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A COMPARATIVE STUDY OF THE INFECTIONS PRODUCED BY INTRAVENOUS INJECTIONS OF TYPHOID, PARATYPHOID A AND B BACILLI IN NORMAL AND IMMUNIZED RABBITS

EXPERIMENTAL TYPHOID-PARATYPHOID CARRIERS. IV

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In order to establish a "carrier state" with absolute regularity, it is unquestionably necessary to obtain as clear a picture as possible of the cycle of events which follows an intravenous injection of gram-negative bacilli in the rabbit. The results of a number of experiments, dealing with the fate and disappearance of the typhoid and paratyphoid bacilli traced from the blood through the various organs are presented in this paper. In subsequent publications of this series, the removal of typhoid bacilli from the blood, their appearance in the bile and urine, the behavior of the leukocytes, etc., will be treated separately. Observations recorded in paper 3 suggested an analysis of the distribution and disappearance of typhoid bacilli in the organs of immunized rabbits. This study was part of a more general analysis of the mechanism of typhoid immunity in the rabbit, guinea-pig, cat, rat and monkey, and was undertaken in the form of a survey to prove or to refute the rather plausible conception of a cellular immunity in typhoid fever.

Observations on guinea-pigs also suggested some experiments with paratyphoid A and B bacilli. It will be demonstrated that paratyphoid A behave like typhoid bacilli in normal and immunized rabbits, while the paratyphoid B bacilli are more rapidly destroyed in the immune than in the normal rabbit. The mechanism responsible for the resistance and the ability of the normal, immune or immunized rabbit to destroy a large number of bacteria of the typhoid-paratyphoid group has been

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investigated in a few preliminary experiments. But interwoven with all these detailed studies are the main questions: Why does the gallbladder become infected? Why does this viscus quite frequently retain the infection? The experiments recorded in this paper contribute some facts to the solution of this problem.

The technical execution of the contemplated extensive experimental program progressed rather slowly on account of the war, but was practically completed, when the studies of Parker and Franke[1] and of Stone[2] appeared in print. The results reported by the latter are contrary to those reported by Parker and Franke; namely, Stone claims to have demonstrated that typhoid bacilli disappear "more quickly from the organs of immune than from normal animals"; while Parker and Franke find "no difference in the bactericidal powers of normal and immune rabbits to intravenous injections of typhoid bacilli." The observations of the latter workers are in accordance with those reported by Bail[3] and our own, although the data presented in their paper, as will be discussed later, are open to serious criticism. On the other hand, we see no justification in the assumption of Stone that the results of Parker and Franke are probably due to the fact "that too many organisms were injected." A careful study and calculation of the figures published shows that Stone injected (with one or two exceptions) one third of a standard blood-agar slant or approximately 460,000 million (?), while Parker and Franke used on the average 39,627,000 (p. 306) to 530-616 million (p. 305) typhoid bacilli. This newly created controversy was investigated and was satisfactorily solved, confirming the findings of Parker and Franke, by a series of additional tests recorded in this paper.

Methods.—The rabbits used in the experiments recorded were all free from coccidiosis; a series in which one animal was found to be infested with these protozoa has been discarded for reasons repeatedly stated in previous papers. With a few exceptions, which are noted, one set of experiments was always conducted on animals of one and the same litter, or a combination of two litters. The latter procedure was necessary only when tests on immunized and normal rabbits were extended over an observation period of 72-96 hours. In order to exclude spontaneous infections with coccidia the litters were raised from does which were known to be healthy, under our own supervision. This likewise excluded to a certain degree the well-known variable resistance to typhoid infections of rabbits of different breeds. Animals of definite age, weight, and of known ancestry were tested with varying amounts of living bacteria. It appears appropriate to emphasize that experimental bacteriologic studies in which the resistance of the animals plays an important rôle should only be conducted on offsprings with a well-known ancestorial history. This fact should govern future experimental work on animals. Some incomplete data at our disposal suggestively indicate that increased resistance to bacterial infections may be inherited; on several occasions it was noted that the offspring of intensively immunized mothers were destroying typhoid bacilli in a shorter time interval than the young of a nonprotected mother. Attention is also called to the family predisposition for urinary infections (Bell and Hartzell[4]).

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[1] Jour. Med. Research, 1919, 39, p. 301.

[2] Jour. Infect. Dis., 1919, 25, p. 284.

[3] Arch. f. Hyg., 1905, 52, p. 272.

The typhoid cultures employed were recently isolated and were either grown on peptic-digest agar or 10% rabbit blood-agar slants. The 18-24 hour old growth was suspended in salt solution strained through cotton, and the number of bacteria per c c of suspension was determined by the dilution plate method. All cultures of one series were made in peptic-digest agar of the same lot. This inexpensive medium surpasses the ordinary veal infusion or meat extract-peptone agar. It is most unwise to express the dosage in slants; uneven growth, as a result of repeated transplanting, etc., produces enormous errors.

In a few experiments a single strain and in others a mixture of 4 to 6 strains was employed. Observations to be reported elsewhere suggested the use of polyhomogenous bacterial antigens. It is selfexplanatory that the immunized rabbits were protected against all the strains included in the test cultures chosen for the infection.

The rabbits were inoculated slowly by various methods specified in the experiments. After varying intervals they were anesthetized with ether, and completely exsanguinated from one or both carotid arteries. The excised organs in toto were placed in Petri dishes and weighed. Pieces of the organs (varying from 0.5 to 6.0 gm.) were then removed, weighed on sterile paper, transferred to sterile mortars and pulped thoroughly with sand. The ground tissues were emulsified in sterile salt solution in the proportion of 1 c c to 100 mg. of tissue. Not infrequently the whole spleen, gallbladder wall, suprarenal, etc., were treated in this manner. The gallbladder wall was carefully dissected from the liver tissue and thoroughly rinsed in salt solution, which was removed by sterile filter paper. The suspensions were prepared without delay after the organs had been excised, and the emulsions were plated in peptic-digest agar in amounts representing from 0.1 to 0.000001 gm. of the organ. Saline was used for the dilution of the emulsion, and by proper shaking it was attempted to obtain a uniform suspension, and a breaking up of the agglutinated bacteria. The plates were counted after 36-47 hours' incubation at 37 C. All figures given in the tables are calculated for 0.1 gm. or 100 mg. of tissue; this number represents not infrequently the average of duplicate or triplicate observations.

Contamination of the plates occurred only in exceptional instances, unless the tissues had been secondarily invaded intra vitam by various types of bacteria. B. bronchisepticus in the lung cultures of rabbits was the only organism which required the use of slide agglutination tests for identification, and it invariably necessitated a repetition of the experiment. Streptococci in the gallbladder or kidney were readily differentiated. All experiments recorded have been performed in duplicate or triplicate, but only one experiment of each series illustrating the result will be given in detail in the tables of this paper.

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[4] Jour. Infect. Dis., 1919, 24, p. 628.

THE LOCALIZATION AND SUBSEQUENT DISAPPEARANCE OF B. TYPHOSUS FROM THE ORGANS OF NORMAL RABBITS INOCULATED BY WAY OF THE BLOOD STREAM

The studies of Wyssokowitsch,[5] Bail,[3] Arima,[6] Bull,[7] and Parker and Franke [1] dealing with the course of a typhoid bacillus infection in rabbits have established the fact that the inoculated bacteria accumulate most abundantly in the liver, next in the spleen and bonemarrow. Destruction of the micro-organisms apparently takes place in the blood of the general circulation, through the action of the leukocytes, or the endothelial cells which line the capillaries most numerously in organs where the bloodstream is slower (Wyssokowitsch). In the majority of instances the foregoing tests were made on rabbits inoculated by the intravenous route and with comparatively small numbers of typhoid bacilli. Moreover, the period of observation was rarely extended over 24 hours, and little or no attention was paid to the gallbladder and its content. It has been pointed out in the preceding paper, and by Nichols [8] that large doses are necessary to ensure gallbladder infections, and that as a rule the injection of bacteria into the portal system produces a higher percentage of persistent carriers than the usual intravenous injection. The purpose of the experiments reported is to verify these claims.

Exper. 1. Relation of the Number of Typhoid Bacteria Injected to the Number of Bacilli Found in the Tissues and Bile Ten Minutes After Inoculations.—Three rabbits of a litter 4½ months old were injected under ether anesthesia with varying numbers of a polyhomogenous mixture of 4 recently isolated strains of B. typhosus (K, B1, B and I). The exsanguination of the animals began at the end of 10 minutes and was completed in 5-6 minutes. The organs were immediately excised and plated. The results are presented in table 1.

Study of the table clearly shows that the intravenous injection of only a large dose of typhoid bacilli leads to an elimination of organisms in the gallbladder or the hepatic duct bile, and that the distribution of the inoculated bacilli in all 3 animals is strikingly uniform. Even after complete exsanguination, the liver, the spleen, the bonemarrow, the lungs, the kidneys, and the gallbladder wall, in the order mentioned, retained the inoculated typhoid bacilli. Two observations are of importance: First, the total number of bacteria recovered as estimated from the blood and tissue cultures was far below the actual number injected. This fact, originally observed by Bull, definitely indicates that inside of 20 minutes in vivo, and inside of 30-50 minutes in vitro—time which must be allowed for the necropsy and the preparation of the cultures—30-50% of the inoculated viable typhoid bacilli are sufficiently injured by phagocytosis or otherwise, or perhaps so completely clumped, that our plating method fails to record the true number present. For the present it is impossible to state which one of the various factors is responsible, but it should be kept in mind that the endothelial cells can be phagocytically active even for a few hours after the death of the animal. Second, the percentage of bacteria accumulating in the liver depends somewhat on the total number of typhoid bacilli inoculated. For example, in rabbit 1378, which received a whole slant, the liver retained 12,870 million bacteria, or 34% of the total number injected; while rabbit 1377, which received 1/10 slant, registered only 859 million, or 22%; and rabbit 1380 (1/100 slant) showed 6.8 million, or 18% of the total. On the other hand, the spleen registered only 0.5% of the actual number injected. The liver, as a whole, however, retained

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[5] Ztschr. f. Hyg., 1886, 1, p. 3.

[6] Arch. f. Hyg., 1911, 73, p. 265.

[7] Jour. Exper. Med., 1914, 20, p. 237; 1915, 22, pp. 475 and 487; 1916, 23, p. 419; 24, p. 25.

[8] Jour. Exper. Med., 1916, 24, p. 502.

TABLE 1

DISTRIBUTION OF B. TYPHOSUS IN THE RABBIT FOLLOWING INTRAVENOUS INJECTION OF VARYING AMOUNTS OF ORGANISMS

Litter: Injected and Exsanguinated Under Ether.

Tissue	Injection of 1 Slant = 38,500,000,000 Organisms	Injection of 1/10 Slant = 3,850,000,000 Organisms	Injection of 1/100 Slant = 385,000,000 Organisms
	Rabbit 1378 (W. 1775)	Rabbit 1377 (W. 1775)	Rabbit 1380 (W. 1575)
Left and central liver lobe	22,100,000	1,750,000	130,000
Right and central liver lobe	16,800,000	1,350,000	136,000
Gallbladder wall	130,000	18,800	2,100
Bile	75 (0.8 c c)	14 (2.0 c c)	0 (1.5 c c)
Spleen	7,600,000	4,020,000	142,000
Bonemarrow	2,700,000	530,000	52,000
Mesenteric lymph nodes	27,000	1,900	25
Lungs	750,000	48,000	>2,000 (few bronchi septicus col.)
Kidneys	156,000	9,800	300
Urine	47 per c c	0	0
Carotis blood	22, and 14,000,000 per c c 18,000,000	1,310,000 per c c	62, and 98,000 per c c 80,000 per c c
Heart blood	11,000,000 per c c	....	32,000
Duodenal content	125 B. typhosus colonies	Sterile: negative for B. typhosus	Sterile
Sacculus rotundatus	....	....	Neg. for B. typhosus
Appendix wall	500 B. typhosus colonies	150 B. typhosus colonies	1 B. typhosus colony

more than 50% of the number of bacteria demonstrated in all the other organs, blood, and skeletal muscles, etc., combined.

It should be pointed out that the distribution of the retained bacteria was uneven in the various portions of the liver, which was probably the result of an uneven dispersion of bacilli in the blood-stream. Furthermore, the gallbladder as an appendix of the liver receives an equal share of the bacteria accumulated in the liver. This statement is supported by the following figures estimated from a number of counts obtained in this and similar experiments:

Rabbit 1.—0.33 gm. of gallbladder wall retain 23.000 B. typhousus.
0.33 gm. of liver retain 18.150 B. typhosus.
Rabbit 2.—0.21 gm. of gallbladder wall retain 19.740 B. typhosus.
0.21 gm. of liver retain 31.000 B. typhosus.
Rabbit 3.—0.27 gm. of gallbladder wall retain 50.760 B. typhosus.
0.27 gm. of liver retain 65.000 B. typhosus.

An intravenous injection of typhoid bacilli into the rabbit will therefore always lead to an invasion of the gallbladder wall. If a sufficiently large dose is given, the clumped bacteria in the capillaries of the wall will probably not remain innocuous, but may lead to changes which favor a chronic infection of the bile and the biliary system. This conception will be discussed in the course of the analysis of additional experiments.

In the kidneys only a small number of bacteria accumulated. It is, however, shown that the injection of a very large dose of typhoid bacilli leads to an elimination of these bacteria by the urine. This phase of the experiment will be taken up later.

This and similar experiments also demonstrated that a very large number of typhoid bacilli cannot be removed readily from the blood-stream inside of 20 minutes. Smears prepared from the heart blood of such animals revealed a few loosely accumulated bacilli, while preparations made from rabbits injected with a small number of typhoid bacilli usually showed no bacteria, or a few clumps of aggregated bacilli. It is, therefore, obvious that the usual mechanism of removal of typhoid bacilli introduced into the circulation of rabbits by clumping, and subsequent phagocytosis in the organs, so ably investigated by Bull,[9] is considerably impaired, when overwhelming doses are used. The phenomenon of "agglutination" in platelets is probably closely connected with the mixing or dispersion of the heavy bacterial suspension with the blood, and the irregular gathering of the organisms in the lung capillaries; but this entire mechanism will be considered elsewhere more in detail. The inability of the choked organs to dispose of the retained typhoid bacilli constitutes an important factor in the formation of foci, which continue to maintain the bacteria in the blood stream.

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[9] Jour. Exper. Med., 1915, 22, pp. 475 and 487.

These experiments also prove that a fairly large dose of over 1,000 million organisms is necessary to produce in the rabbit an elimination of bacteria in the bile after an ear vein injection. It is a debatable question whether the appearance of typhoid bacilli in the hepatic or cystic bile of rabbits, subsequent to an intravenous injection, can really serve as an indication that such an animal will become a chronic carrier or develop a cholecystitis. It is, however, certain that the body does not free itself by excreting the living bacteria in the bile and urine. The number of organisms so eliminated is, in proportion to the inoculum, very small; in our case, one five-millionth of the infecting dose of bacilli. Assuming for the present that the elimination of bacteria with the biliary secretion alone is responsible for a persistent gall-bladder infection, it is not surprising to record that Nichols[8] Tanabe and Takeuchi[10] and others noted a higher percentage of carriers in rabbits infected by way of the mesenteric than the ear vein. In their tests more organisms appeared in the bile after portal vein injections than after ear vein injections. We have compared the two methods of infection in several experiments, but have been unable to confirm these claims, as is shown by the results reported in the following:

Exper. 2. The Fate of Typhoid Bacilli Introduced Into the Circulation by Way of the Portal Vein and by Way of an Ear Vein. Comparison Between Intravenous and Subcutaneous Mode of Infection.—Two rabbits (1343 and 1342) of the same litter, 211 days old, received injections, using the ear vein, with 5,000 million typhoid bacilli strain K., and one rabbit (1349) received the same amount subcutaneously. Two rabbits (1344 and 1345), also of the same litter, were laparotomized under ether anesthesia and the bacilli were introduced into the circulation by way of a mesenteric vein. The animals were exsanguinated and their organs plated at the intervals noted in the table.

Study of table 2 shows that the distribution of the injected typhoid bacilli was, with 2 exceptions, the same for the 4 rabbits receiving the infective dose by way of the blood stream. Comparatively few bacteria appeared in the bile 10 minutes after the injection; the rabbit injected in the ear vein showed the largest number. The liver of this animal removed from the blood stream 9% of the inoculated organism, while

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[10] Mitt. a. d. Mediz. Gesellsch. z. Osaka, 1910, 9, refer.; Centralbl. f. Bakteriol. I. Ref., 1911, 50, p. 294.

TABLE 2

Fate of B. Typhosus on Intraportal and Intravenous Inoculation Compared by the Subcutaneous Method of Injection

Litter Experiment: Intravenous injection of 5,000,000,000 organisms.

Tissue	10′	10′	24 Hours	24 Hours	24 Hours
	Intraportal Injection: 1344	Intravenous Injection: 1343	Intraportal Injection: 1345	Intravenous Injection: 1342	Subcutaneous Injection: 1349
Left and central liver lobe	650,000 (800,000)	810,000 (400,000)	2,400 (3,000)	6,000 (7,400)	0
Right and central liver lobe	550,000 (860,000)	660,000 (400,000)	2,000	6,720 (11,000)	0
Bile	5 (0.4 c c)	60 (0.2 c c)	0 (1 c c)	0 (0.8 c c)	No gallbladder
Gallbladder wall	9,400	1,690	2	128	
Spleen	750,000	2,900,000 (2,310,000)	4,410	1,470	0
Bonemarrow	30,000	26,000	23,000,000	410 (380)	0
Mesenteric lymph nodes	380	120 (72)	8	20	0
Lungs	2,500	6,600	10	136	0
Kidneys	450+	590 (420)	0	7	0
Urine	0 (1 c c enriched 0)	....	0 (cocci)	0 (cocci)	0
Testes	45	....	....	....	....
Carotis blood	46,000 per c c	125,000 per c c	2 per c c	3 per c c	0
Heart blood	8,860 per c c (22,300; 840; 5,000)	18,500 per c c (37,000; 2,900; ....)	1 per c c	0	0
Brilliant Green Eosin Plates:					
Duodenum contents	Sterile	1 colony B. typhosus	Sterile	Sterile	Sterile green plates
Duodenum wall	4 colonies pure	150 B. typhosus pure	7 colonies B. typhosus	Negative for B. typhosus	Sterile green plates
Jejunum contents	Sterile	2 B. typhosus pure	Sterile	Negative for B. typhosus	Negative for B. typhosus
Jejunum wall	4 colonies pure	300 B. typhosus + coli	Sterile	Negative for B. typhosus	Negative for B. typhosus
Ileum contents	B. coli	B. aerogenes	1 B. typhosus +++	Negative for B. typhosus	Negative for B. typhosus
Ileum wall (sacculus rotundatus)	3 colonies B. typhosus ∞ B. aerogenes	3 colonies B. typhosus + B. aerogenes	800 B. typhosus +++	Negative for B. typhosus B. coli	Negative for B. typhosus
Appendix contents	B. coli aerogenes	1 colony B. typhosus	1 B. typhosus	Negative for B. typhosus B. coli	
Appendix wall	3 colonies B. typhosus	B. coli aerogenes	55 B. typhosus +++	Negative for B. typhosus B. aerogenes	

the intraportal injected animal accumulated only 6% of the total dose. At the end of 24 hours the gallbladder bile was sterile in the remaining 3 animals. More bacilli accumulated in the lung and spleen of the animal injected by way of an ear vein than in the one infected through a portal vein. It is generally known that after an intravenous injection bacteria accumulate in the lung capillaries, and that intraportal injections lead to a saturation of the vessels of the liver. Rabbit 1345 was slightly toxic, which explains the high count in the bonemarrow, as has already been pointed out by Parker and Franke.[1] Microscopically, this tissue showed necroses, innumerable nests of free and clumped typhoid bacilli, and a marked absence of regeneration of polymorphonuclear leukocytes. The saline extract of the liver of this animal was atoxic. Of some interest were the bacterial counts obtained from the intestinal wall of rabbit 1345, in comparison with rabbit 1342. Numerous hemorrhages in the sacculus rotundatus and the appendix wall indicated vascular lesions in the intestinal mucosa and submucosa. The cultures proved the cause of these hemorrhages to be nests of typhoid bacilli, and supported the observations of Ribadeau-Dumas and Harvier,[11] and of Chirolanza,[12] that the intestinal content of rabbits can become infected from bacterial emboli and hemorrhages in the intestinal wall. It is obvious that the mesenteric vein inoculation was particularly conducive to the localization of clumped bacilli in the capillaries of the intestines. Under particularly favorable conditions (large infective dose) it is readily possible that the gallbladder wall may become similarly infected. Our particular experiment fails, however, to confirm this conclusion. It is not unlikely that postoperative changes (slight peritonitis, biliary stasis, etc.), which develop as a result of the technic employed to introduce typhoid bacilli by way of the portal system, favor the localization of bacteria in the gallbladder, and they may in part explain the results obtained by Nichols.[8] The intoxication of rabbit 1345, which was the largest animal of the series, points toward the explanation offered. Moreover, in our experience postoperative starvation is conducive to intoxication, and the formation of foci in the bonemarrow and biliary system. On the other hand, our results differ in no respect from those reported by Carmichael [13] and by Else.[14] Both experimenters show that organisms injected into the portal vein do not readily reach the gall-

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[11] Compt. rend. Soc. de biol., 1910, 69, p. 181.

[12] Ztschr. f. Hyg. u. Infektionskrankh., 1909, 62, p. 11.

[13] Jour. Path. & Bacteriol., 1903, 8, p. 276.

[14] Surg., Gynec. & Obst., 1910, 11, p. 470.

TABLE 3

FATE OF B. TYPHOSUS IN NORMAL RABBITS

Rabbits of the same litter, 5 months old; exsanguinated following injection. Injected intravenously: 4,300,000,000 B. typhosus "Kearney."

Tissue	10′	60′	1 Day	2 Days	3 Days	4 Days	5 Days
	Rabbit 1225	Rabbit 1222	Rabbit 1219	Rabbit 1220 C.	Rabbit 1221	Rabbit 1223	Rabbit 1224
Right liver lobe..........	1,610,000	420,000	700,000	2,320,000‡	180 (139)¶	370	......#
Left liver lobe..........	1,130,000	550,000	10,000?	5,640,000	700	112 (106, 200)	2,133
Center liver lobe..........	1,620,000	380,000	50,400	36,000§	130 (180)	111 (105, 200)‖	56,000
Gallbladder wall..........	300	310	225,000	64,000	792,000	508,000	112,800
Bile..........	4 per c c	1?	D† = sterile; enriched: 0	19,000,000 per c c (0.4 c c)	10,550,000 per c c	8,000,000 per c c	2,000,000 per c c
Spleen..........	1,280,000	370,000	6,700	1,200	118	245	385
Left bonemarrow femur..	221,000	125,000	1,400	150	13	4 (10)	7
Right bonemarrow femur	190,000	170,000	1,200	330	37	12	3
Mesenteric lymph nodes...	250	24	270	67	175?	60	10-21
Lungs..........	13,000 right	....	{300 right lung {290 left lung}	70	6	0	0
Left kidney..........	5,700	52	11	0	0	1	0
Right kidney..........	....	10	24	4	0	2	0
Femoral muscle..........	250	....	900?	0	0	0	0
Thymus..........	....	....	124	70	4	....	....
Carotis blood..........	1,000,000 per c c	<100*	1,300 per c c	100 per c c	7 per c c	1 per c c	0
Right ventricle..........	380,000	....	....	....	....	Sterile	Sterile
Duodenum..........	6 colonies per loop	0	∞ B. typhosus per loop	∞ B. typhosus per loop	∞ B. typhosus	∞ B. typhosus	∞ B. typhosus per loop
Sacculus rotundatus......	0	0	0	∞ B. typhosus per loop	∞ B. typhosus	∞ B. typhosus	30 B. typhosus per loop
Stool in rectum..........	0	0	0	50 B. typhosus per loop	∞ B. typhosus	2 B. typhosus per loopfull	2 B. typhosus per loop
Urine..........	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile

* Dilution too high. † D = direct quantitative plating. ‡ Kidney lobe: 760,000. § Around gallbladder: 630,000.
¶ Kidney lobe: 180. ‖ Liver around gallbladder: 1,850 + 3,600. # Kidney lobe of liver: 92.

bladder. *From an experimental standpoint the mesenteric vein injection offers, therefore, no advantages over the ear vein injection.*

A subcutaneous injection of a large dose of typhoid bacilli fails to cause a general infection. At the end of 24 hours a small number of the introduced organisms can be found at the site of inoculation, while the viscera by ordinary plating procedures are found to be sterile. This experiment confirms similar ones reported by Doerr.[15]

The next important step in the analysis of the fate of typhoid bacilli inoculated into rabbits consisted of culturing the tissues of nonimmunized animals over a period of at least five days. Such experiments demonstrated the ability of certain organ tissues to dispose of the accumulated bacteria and supplied data which indicated that a prolonged infection of the bile was associated with an involvement of the liver and the gallbladder wall. One of these instructive experiments is reported herewith.

Exper. 3. The Fate of Typhoid Bacilli in Nonimmunized Rabbits.—Seven rabbits of one litter, with an average weight of 1630 gm., 129 days old, were inoculated into an ear vein with 4,300 million typhoid bacilli strain K., grown on peptic-digest agar. The animals were exsanguinated after 10 and 60 minutes, and after 1, 2, 3, 4 and 5 days, and the excised tissues plated. The results are shown in a concrete form in table 3.

It will be noted from table 3 that within 10 and 60 minutes, even 24 hours, the cystic bile contained very few typhoid bacilli, while the gall-bladder wall recorded for rabbit 1219 a very high count. This observation, together with other data to be reported elsewhere, is in our opinion conclusive evidence that a wall infection frequently precedes the invasion of the bile. Erosion of the capillaries of the gallbladder wall occurred in rabbit 1221. This animal showed a blood-tinged cystic bile with leukocytes and fibrin flakes; the wall itself exhibited patches of necroses and mucosal and subserous hemorrhages. In the other rabbits, aside from a high bacterial count, pronounced vascular changes were not evident, but the liver tissue adjacent to the gallbladder registered a large number of viable bacteria. In the experiment under consideration, at least 48 hours elapsed before the cystic bile was heavily infected, although the hepatic duct bile probably contained typhoid bacilli much earlier as innumerable colonies were present on the plates prepared from the duodenum of rabbit 1219. It may be mere coincidence, but the gradual diminution in the number of bacilli per c c of bile in the rabbits sacrificed on the 4th and 5th day is noteworthy, and may be explained by the more active evacuation of the stagnant biliary secretion, stimulated by the digestive activities of the intestinal tract, which was seriously impaired during the intoxication of the animals in the first 3 days following the injection.

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[15] Centralbl. f. Bakteriol., I. O., 1905, 39, p. 624.

Aside from the points just mentioned, the results recorded for this and similarly conducted experiments did not as a whole differ from those reported by various writers repeatedly mentioned. The liver retained, 10 minutes after the injection, about 20% of the total number of bacteria introduced into the blood stream, while all the other organs, particularly the kidneys, lymph nodes, muscle, etc., retained very few. The bactericidal action of the serums of rabbits 1225 and 1222 was comparatively low; 1 c c of serum destroyed in 5 hours only 10,000 typhoid bacilli stain K. This may in part explain the high bacterial count of the blood, and the fact that clinically the animals of this series were in comparison with others only slightly intoxicated. This observation is also supported by the low bonemarrow counts. The speed of destruction is influenced by the resistance of the rabbit, and the number of bacteria inoculated, as is shown by the fact that the liver in rabbit 1222, at the end of one hour following the inoculation, still retained 5% of the total number of bacteria, or about one-fourth of the number of typhoid bacilli which originally lodged in this organ.

Aside from the liver, the gallbladder and its contents, the spleen and bonemarrow may retain viable bacteria at a period when the blood is already sterile, and may remain the site of vegetating typhoid bacilli for from 20-30 days. As a rule, the spleen destroys typhoid bacilli more slowly than the liver, a fact already noticed by Werigo.[16] Simultaneously with the disappearance of the germs from the blood, which depends on the bactericidal substances evolved by the tissues and the number of bacilli injected, the kidneys and lungs are found free, while the mesenteric lymph nodes, probably one account of secondary invasion through lacteals of the intestines, remain more or less infected.

These experiments conclusively demonstrate that the typhoid cholecystitis in normal, healthy rabbits is probably not infrequently the result of capillary bacterial emboli in the gallbladder wall, and that a pericholangitis, or a hepatitis, as has been stated by E. A. Graham [17] is a rather constant accompaniment of this process. This phase of our problem will, however, be considered in the next paper of this series.

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[16] Ann. de l'Inst. Pasteur, 1892, 6, p. 478.

[17] Surg., Gynec. & Obst., 1918, 27, p. 521.

A COMPARISON OF THE FATE OF TYPHOID BACILLI IN NORMAL AND IMMUNIZED[18] RABBITS

It has already been stated that Bail, Parker and Franke, and Stone studied the bactericidal powers of normal and immunized rabbits infected by intravenous injections. It has also been pointed out that the results of these workers were obtained by various procedures and that they are not strictly comparable. Bail, for example, worked with rabbits, which were passively immunized by the injection of immune serums. He stated that he encountered considerable difficulty in obtaining uniform results. Parker and Franke prepared their immunized animals with killed and living typhoid bacilli, while Stone inoculated her rabbits with dead bacilli only. Parker and Franke tested their animals with a comparatively small number of typhoid bacilli, while Stone used the profuse growth of blood-agar slants. It naturally suggested itself to investigate whether the various results recorded by these workers were attributable to these differences in technic or to other unknown factors. Our experiments were conducted with these problems in mind, and covered, therefore, three phases: 1. What is the fate of typhoid bacilli intravenously injected into rabbits immunized by dead or living vaccines? 2. Are typhoid bacilli grown on peptic digest agar less readily destroyed by the bactericidal powers of the rabbit's blood and tissues than those grown on rabbit blood agar.[2] 3. Does the dosage of bacteria injected materially influence the end result, and does the immunized animal really destroy typhoid bacilli better and in a shorter time interval than the normal rabbit? The respective experiments to answer these questions were extensive, and our conclusions were based on several hundred animals. For the sake of brevity an example of each type of test will be selected for consideration and discussion.

Exper. 4. The Fate of Typhoid Bacilli in Normal Rabbits and in Those Immunized by Heat-Killed Tricresolized Vaccines Tested by a Moderately Large Number of Bacteria.—Five rabbits of two litters of 11 animals, with an average weight of 2,090 gm., and about 145 days old, were immunized by six intravenous injections of polyhomogenous heat-killed, tricresolized vaccines (13 and 14). Forty days after the last injection these rabbits, together with 6 control animals, of an average weight of 2297 gm., were infected by an intravenous injection of 3,300 million B. typhosus strain K. The rabbits were exsanguinated in pairs after 10 minutes, 5 hours, 1, 2, 4 and 6 days, and the excised organs plated. The counts of viable bacteria are shown in table 4.

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[18] Rabbits are immune against the disease typhoid fever, but they can be immunized against the B. typhosus. In order to emphasize this difference the term immunized instead of immune is chosen.

TABLE 4

The Fate of Typhoid Bacilli in Normal Rabbits and in Those Immunized by Heat-Killed Tricresolized Vaccines Tested by a Moderately Large Dose of Bacteria

Tissue	10′	10′	5 Hours	24 Hours	24 Hours	48 Hours	48 Hours	96 Hours	96 Hours	144 Hours	144 Hours
	Immune Rabbit 1283	Normal Rabbit 1284	Normal Rabbit 1285	Immune Rabbit 1274	Normal Rabbit 1278	Immune Rabbit 1275	Normal Rabbit 1279	Immune Rabbit 1276	Normal Rabbit 1280	Immune Rabbit 1277	Normal Rabbit 1281
Agglutination	1:2000	<1:10	<1:10	1:10,000	<1:10	1:20,000 +++	<1:10	1:10,000– >1:20,000	<1:10– 1:8,000 +++	1:8,000– >1:20,000	<1:10– 1:4,000
Liver, left and central	910,000	940,000	4,000-8,000	280	450	210	68-90	132	5,800	10	0
Liver, central and right	1,080,000	830,000	4,500	230	380	270	80	200-260	6,400	8–20	2
Gallbladder wall	4,100	730	9	0	2	0	0	0	82,000	0	0
Bile	11 (0.6 c c)	0 (also enriched)	169 (1.6 c c)	0 (1.8 c c)	0 (0.5 c c)	0 (0.6 c c)	0 (0.9 c c)	0 (1 c c)	103,500,000 p. bile	0 (1.3 c c)	0
Spleen	360,000	230,000	37,000	>3,500	200	201-500-350	104	1,120-1,000	3 (enriched 0)	138-240	0
Bonemarrow pooled	>130,000+	3,100-5,100	>50,000	1,100	50-80	70	6-8	54	1	4	0
Mesenteric lymph nodes	280	60	90	2	52	82	6	105-160	0	1	0
Lungs	12,000	650	200-1,000	70-100	20	0	0	130-200	0	1	0
Kidneys	540	55	17	1	0	0	0	0	910	2,700	0
Urine	0	....	0	260{210 310	0	......	0	0	++	33 (in 1 c c)	0
Heart blood	0?		100	......	......	0	0 R. & L. V.	0	0	0	0
Carotis blood	750	100 2,130	400	0	?	1 in 4 plates	1	1 in 3 plates	1 in 3 plates	2 in 3 plates	0
Whole blood clotted	230-40-20-72 (5 hours)	10-20-0 (5 hours)	40-20-0 (5 hours)	0	6						

96 Hours	
Normal Rabbit 1280	
Duodenum	∞ B. typhosus
Jejunum	∞ B. typhosus
Colon	∞ B. typhosus
Sacculus rotundatus	B. typhosus
Stool from rectum, direct	Negative
Bile enriched	B. typhosus
Biliary passages	4,040,000
Liver around gallbladder	21,200

144 Hours	
Immune Rabbit 1277	
Duodenum	Negative for B. typhosus
Jejunum	Negative
Ileum	Negative
Sacculus rotundatus	Negative
Cecum	Negative

144 Hours	
Normal Rabbit 1281	
Duodenum	Negative
Jejunum	Negative
Ileum	Negative
Sacculus rotundatus	Negative
Cecum	Negative

Table 4 shows that the ratio of distribution of the typhoid bacilli in the organs of immunized rabbits is practically the same as in the normal animal. In this particular experiment the spleen, bonemarrow and lung of the immunized rabbit sacrificed 10 minutes after the injection, retain more bacteria than the normal. In other experiments this difference is constant only for the lung: This organ may in animals with an acquired immunity seize from 3 to 200 times as many bacteria as that of the normal rabbit. Rather constant is the observation that the splenic tissues of the vaccinated animals retain the typhoid bacilli injected for a longer period, and in somewhat greater number than the normal. These are the only striking differences between the normal and immunized rabbit which have occurred regularly in similarly conducted experiments. The blood serums of the normal rabbits of the two litters exhibited exceedingly high bactericidal properties (see figures on whole blood clotted of rabbit 1284 and rabbit 1285), which in part explains the disappearance of the injected bacteria from the blood stream, irrespective of their low agglutinin content. It is noteworthy that on the sixth day the tissues of normal rabbit 1281 were, with exception of the liver, found sterile, while the immunized animal (rabbit 1277) of the same litter and period of infection, showed typhoid bacilli in most of the organs and even in the blood. The latter finding is probably connected with the persistence of viable organisms in the spleen.

Localization in the gallbladder took place in only one animal (1280). The inflammatory reaction of the gallbladder wall was severe. Indeed, fibrin deposits connected the serosa of the viscus with the stomach wall. An edematous infiltration extended along the cystic duct to the common and to the hepatic biliary passages. Flocculent, slimy débris blocked the common duct and in part explained the high bacterial count of the bile removed from the gallbladder. Judging from these and similar findings, one is again inclined to attribute the cholecystitis to a primary infection of the wall with subsequent invasion of the bile. Attention is also called to the presence of typhoid bacilli in the urine of 3 animals (rabbits 1274, 1280, and 1277). In 2 rabbits this persistence of organisms is associated with a localization of bacteria in the kidneys. Similar observations have indicated that the presence of typhoid bacilli in the urine is frequently characteristic for animals of the same litter, and we therefore suspect an hereditary susceptibility of these organs. Moreover, the typhoid bacilli can multiply in the urines of some rabbits, while in others the findings of these germs is always closely connected with foci in the kidney parenchyma.

The method of immunization, whether accomplished with living or dead organisms, has no influence on the destruction of typhoid bacilli in the blood and tissues of the protected rabbits, as is illustrated by experiment 5, which is again chosen from a number of similar tests.

Exper. 5. The Fate of Typhoid Bacilli in Rabbits Immunized With Living Vaccines and Tested by the Injection of a Moderately Large Dose of Bacteria.— Three members of a litter of 7 rabbits, with an average weight of 1,700 gm., and 132 days old, were immunized according to the method of Bull[19] with a mixture of 4 strains (Bl., B., K. and I.) of living typhoid bacilli. Twenty-eight days after the last injection these rabbits, together with the 4 controls of the same litter, were injected intravenously with 4,100 million of a B. typhosus mixture (strain Bl., B., K., and I.). The distribution and localization of the injected bacteria were studied in one normal rabbit. The remaining 6 animals were sacrificed in pairs after 8, 24, and 72 hours, and the organs plated. The observations are summarized in table 5.

A study of table 5 shows that the serums of the nonimmunized members of this litter destroyed typhoid bacilli rather slowly. The immunization rendered the blood nonbacteriolytic in the test tube, and yet in 2 rabbits killed on the 24th and 72nd hour after the injection, the blood stream harbored fewer organisms than in the normal animals (3 and 1 to 44 and 75). On the other hand, the splenic pulp of the immunized rabbit retained from 2 to 20 times as many bacilli as the normal. The lung tissue of the immunized animal retained on the 8th hour 3 times as many bacteria as its liver, and 60 times as many as the lung of the normal control rabbit. Gallbladder infections resulted in only one normal animal. The number of organisms determined in 1.6 c c of bile was 490; the gallbladder wall registered 31 colonies per 100 mg. of tissue. This particular animal showed numerous infarcts in the liver, and in comparison with other rabbits it was obvious that the presence of a small number of typhoid bacilli was probably the result of biliary elimination from these liver foci. Both the normal and immunized rabbits sacrificed on the 8th hour had bacilli in the bile (10 and 36 per 1 c c). This and similar observations indicate that the mere presence of typhoid bacilli in the gallbladder bile does not lead to a prolonged infection of this viscus.

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[19] Jour. Exper. Med., 1916, 23, p. 419.

TABLE 5

THE FATE OF TYPHOID BACILLI IN RABBITS IMMUNIZED WITH LIVING VACCINES AND TESTED BY THE INJECTION OF A MODERATELY LARGE DOSE OF BACTERIA

Tissue	10'	8 Hours	8 Hours	24 Hours	24 Hours	72 Hours	72 Hours
	Normal Rabbit 1327 B	Immune Rabbit 1327	Normal Rabbit 1329	Immune Rabbit 1328	Normal Rabbit 1332	Immune Rabbit 1330	Normal Rabbit 1331
Agglutination............	<1:10	1:10,000	1:10	1:8,000	1:10	1:8,000-10,000	1:60–100
Left and central liver.....	780,000	42,000	60,000	1,360	7,900	62	780 ⎫ In-
Right and central liver....	750,000	36,000	120,000	1,460	8,100	46	1,200 ⎭ farcts
Gallbladder wall..........	5,500	180	220	0 (cocci)	10	0 (cocci)	31
Bile........................	36 per c c (1.0 c c)	10 per c c (1.1 c c)	0 (1.2 c c)	0 (0.8 c c)	0 (1 2 c c)	0 (1.4 c c)	490 per c c (1.6 c c)
Spleen.....................	1,610,000	20,000	1,100	4,760	4,100	6,160	2,500
Bonemarrow..............	364,000	37,000	40,000	2,560	8,000	20	190
Mesenteric lymph nodes...	1,700	152	80	152	95	160	300
Lungs....................	10,000	107,000	1,680	750	300	2 (B. bronchi-septicus)	1 (B. bronchi-septicus)
Kidneys..................	13,200	2,800-3,200	170	19	10	<10	2
Urine....................	0 (3)	0	0 (3)	0 (3)	0 (1)	0 (3)	3,450,000 per c c
Thymus..................	1,050	....	....	....	....	....	....
Carotis blood.............	1 2,325,000 per c c 11 1,620,000 per c c	1 265 per c c II 225 per c c	I 105 per c c II 85 per c c	I 11 per c c II 4 per c c	I 44 per c c II 39 per c c	0, 0, 1 per c c	I 74 per c c II 56 per c c
Heart blood..............	....	120 per c c	210 per c c	3 per c c	34 per c c	0 per c c	75 per c c
Duodenum................	1 colony of B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus
Jejunum.................	....	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus
Ileum.....................	....	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus
Cecum.....................	....	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus
Appendix wall............	2 colonies	61 B. typhosus colonies	Negative for B. typhosus	Negative for B. typhosus	1 B. typhosus	Negative for B. typhosus	Negative for B. typhosus
Appendix contents.......	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	2 B. typhosus ulcer in colon ∞ B. typhosus	Negative for B. typhosus	Negative for B. typhosus

A bacilluria of 3,400,000 typhoid organisms per c c with a few bacteria in the kidneys (2 per 100 mg.) was noted in a normal rabbit killed on the third day. The liver of the normal rabbit killed on the 8th hour contained toxins demonstrable by the method of Parker, while the saline extract of the same organ of the immunized animal was innocuous. On several occasions we tested the fate and destruction of typhoid bacilli in rabbits which were gallbladder-typhoid carriers. The removal of the organisms from the blood by the tissues, and their bactericidal action proceeded in these animals in an identical manner, as observed in control rabbits studied at the same time under identical conditions.

The experiments of Parker and Franke were conducted on rabbits immunized with living typhoid bacilli, while Stone tested animals vaccinated with heat-killed preparations. The only additional differences in the experiments of these workers was the use by Stone of typhoid bacilli grown on rabbit-blood agar slants. Typhoid bacilli acquired on this medium, particularly when transplanted at regular intervals of from 24-48 hours, certain properties which apparently are conducive of gallbladder infections. Physical, as well as biologic factors, which we have not been able to study with the desired thoroughness, are probably responsible for these properties. The growth energy of B. typhosus is enhanced on blood mediums and with it also the pathogenic powers, particularly the parasitic function. This applies in a lesser degree to the toxicogenic properties. In many respects this behavior of typhoid bacilli frequently transferred on blood mediums is analogous to the changes in virulence observed by Wadsworth and Kirkbride[20] for the pneumococcus. From several experimental series in each of which 2 normal rabbits were infected with typhoid bacilli grown on rabbit-blood agar (35 generations) and 2 others with the same number of bacteria reared on veal-infusion agar (same number of generations), it must be concluded that no difference in the distribution and localization or destruction of the germs is demonstrable. Toxic symptoms and a high bonemarrow count at the end of the 24th hour were, however, noted in several animals injected with the veal-agar growth. These observations suggest a number of tests on immunized rabbits and one experiment of a series of 3 is chosen for discussion.

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[20] Ibid., 1918, 28, p. 791.

TABLE 6

The Fate of B. Typhosus Grown on Rabbit-Blood Agar in the Immunized and Normal Rabbit

Tissues	10'	24 Hours	24 Hours	96 Hours	96 Hours	120 Hours	120 Hours
	Normal Rabbit 1595 o+	Immune Rabbit 1646 o	Normal Rabbit 1522 o+	Immune Rabbit 1591	Normal Rabbit 1686*	Immune Rabbit 1589	Normal Rabbit 1593
Agglutination titer	1:10++	1:40,000	1:10	1:60,000+++	1:40+++	1:40,000+++	1:800+++
Left and central liver	1,200,000	840	300	30,000	800,000	60,000	1,600
Right and central liver	2,000,000	760	1,210	1,100	840,000	50,000	630
Bile	1,334 (0.5 c c)	0 (enriched +) 0.05 c c	0 (1.2 c c)	1,420,000 (0.9 c c)	960,000,000 per 0.8 c c	58,000,000 per 0.7 c c	50,000 per 0.8 c c
Gallbladder	7,200	10	0 difco streptococci	19,000	5,500,000	230,000	44,000
Spleen	900,000	1,500	400	24	17,000	1,600	410
Bonemarrow	80,000	5,500	5,000	36	6,000,000	24	58
Mesenteric lymph nodes	15,000	17	18	22	....	3	21
Lungs	22,000	7,000	3,000	250	1,800	150	7
Kidneys	62,000	9	3	180	267,000	0 (enriched ++)	12,000
Urine	3, 2 per c c	0, 0	169 per c c	0: 0	10	0	66,000 per c c
Carotis blood	4,800,000; 6,000,000	35; 20; 14; 20; 23; 14	76; 65; 140; 55; 90	0; 0; 0	....	0; 0; 0; 0; 0; 0; 0	0; 0; 1; 0; 0; 1; 0
Heart blood, left vent.	4,900,000	12: 10	66; 40	0; 0; 0	8 partially coagulated blood	0; 0; 0	3; 0; 0
right vent.	3,000,000	20; 14	40; 130; 90	0; 0; 0	....	0; 0; 0	0; 0; 1
Duodenum	6 colonies pure	Negative for B. typhosus	Negative for B. typhosus	∞ B. typhosus colonies	∞ B. typhosus colonies	5 B. typhosus colonies	∞ B. typhosus
Sacculus rotundatus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	1 B. typhosus colony	∞ B. typhosus colonies	8 B. typhosus colonies	Negative for B. typhosus

* Died shortly before cultures were made.

Exper. 6. The Fate of B. Typhosus Grown on Rabbit-Blood Agar in the Immunized and Normal Rabbit.—Three rabbits of a litter of 7 animals, with an average weight of 2,500 gm., and about 170 days old, were immunized with a mixture of 7 strains of heat-killed and living typhoid bacilli in a similar manner as that described in exper. 5. Fifteen days after the last injection these animals, in addition to the remaining 4 members of the litter, were infected by an intravenous injection of 4,300 million of a polyhomogenous saline mixture of 7 typhoid strains grown on 10% rabbit-blood agar. The cultural findings are shown in a concrete form in table 6.

Attention is called to the fact that the immunized and normal rabbits exhibited pronounced localization of the typhoid bacillus in the gallbladder and bile 96 and 120 hours after the introduction of the infective dose, while the animals killed on the 24th hour gave sterile cultures from this viscus. These observations indicate that blood-agar cultures are suited for the production of biliary infections, but they also confirm the conclusion reached from exper. 3, that the bile or the gallbladder does not necessarily become infected in the first 24 hours. It may also be assumed that elimination of typhoid bacilli by the hepatic duct bile in the first 10 minutes does not necessarily lead to a prolonged infection of this viscus. The evidence in this and similar experiments is decidedly in favor of the conception that, in the rabbit intravenously inoculated, not infrequently an infection of the gallbladder wall, or extensive liver lesions, initiate the invasion of the bile. It should, however, be emphasized that in this respect no difference exists between the immunized and normal rabbits. Anatomically, hemorrhages and even slight erosions of the gallbladder mucosa are distinctly visible; every rabbit shows, also, extensive liver necroses. In rabbit 1589 an obstruction of the common duct produced a uniform dilatation of the bile ducts which explained the high bacterial count of the liver tissue, while in rabbit 1593 the washed gallbladder wall registered practically the same number of typhoid bacilli as 0.8 c c of bile.

The bacterial count of the bonemarrow of rabbit 1686 indicates the existence of a focus, which led, directly or indirectly, to the intoxication and to the death of the animal. The high bacterial counts of the tissues must be partially attributed to a multiplication of the typhoid bacilli in the blood stream during a rather prolonged agonal stage of the disease, and partially to the fact that toward the termination of the infection the organs, like the liver, the spleen and the lungs, primarily engaged in the destruction of the bacteria, are overwhelmed with organisms brought to them from the bonemarrow foci. It is known from the statements already made that 3-4 days usually elapse before the blood of the normal, and frequently also the immunized, rabbits is found sterile. In our experience with a considerably larger number of animals than reported by Stone, we have been unable to confirm her conclusions that the blood of typhoid immunized rabbits sterilizes itself in 18 hours. Heart-blood cultures, of course, should be chosen for this comparison. In rabbits showing extensive liver or splenic foci the carotid blood toward the end of the exsanguination may record a few typhoid bacilli. Furthermore, sterility of the blood is in no way dependent on its content of immune agglutinins or other antibodies. Rabbits immunized with sensitized bacteria, as a rule, lack agglutinins, although they are similarly resistant to an infection as are rabbits protected with living bacteria.

Most of the rabbits in this experiment possessed at necropsy a distinct splenic tumor, and this tissue in the immunized rabbits retained slightly more bacilli per 100 mg. of organ than the normal ones. For reasons already given, the pair of rabbits killed on the 96th hour cannot be included in these deductions.

Localization in the kidneys, early elimination, and even persistence of the typhoid bacilli in the urine, were characteristic for this litter. The latter condition might be due in part to a change in the reaction and composition of the urine. Complete disappearance of the urinary carbonates and a distinct reduction in the alkali blood reserve occurred in the rabbits with urinary infections.

Briefly summarized, it can be stated that the intravenous injection of normal and immunized rabbits with typhoid bacilli grown on rabbit-blood agar failed to demonstrate differences in the destruction and the distribution of the bacteria. It was, however, noted that localization in the gallbladder and bile 96-120 hours after the injection occurred more readily with moderately heavy suspensions prepared from rabbit-blood agar slants than with those cultivated on peptic-digest agar.

In accordance with the plan of our investigation it was necessary to verify the interpretations of Stone: Can an immunized rabbit dispose more rapidly of a small number of typhoid bacilli than a nonimmunized one: and are the results published by Parker and Franke really explained by the fact that these workers employed as an infective dose less than 1 billion bacteria? In three series of experiments these questions were investigated. The number of organisms injected varied from 215-562 million bacteria. Litters of different ages were chosen, and it was conclusively demonstrated that no differences existed in the normal or immunized rabbits, either in the destruction or in the localization of such numbers of typhoid bacilli. Two hundred and fifteen million were disposed of in a shorter time interval than 562 million, but the immunized animal differed in this respect in no way from the normal. Moreover, the variable resistance of different litters, sometimes influenced by B. bronchisepticus infections, was more readily demonstrable following the injection of small doses of typhoid bacilli, but in no case were these variations greater in immunized than in normal animals. The same deduction applies to the apparent differences depending on the toxicity or virulence of the individual typhoid strains. The correctness of these conclusions is illustrated by the description of one experiment.

Exper. 7. The Fate of a Small Dose of B. Typhosus in Rabbits Immunized With Dead and Living Organisms.—Three rabbits of one litter and 3 of another, all 197 days old, and of an average weight of 1,991 gm., were immunized either by Bull's method with a polyhomogenous mixture of typhoid bacilli, or by 6 injections of a heat-killed, tricresolized vaccine. Seventeen days after the last injection, these rabbits, together with 3 controls (members of the litter immunized with dead bacteria) were injected with 350 million typhoid bacilli (7 strains). The cultivating of the organs was conducted as usual. Table 7 presents the results in concrete form.

The figures presented in table 7 seem to indicate that the number of typhoid bacilli inoculated has no appreciable influence on the mechanism responsible for their progressive destruction in the rabbit body. It may be mere coincidence, but in this and similar experiments, it was noted that frequently the normal rabbit sterilized itself in a shorter time interval than the immunized animal. The bacterial counts of the spleen and bonemarrow should be chosen for such comparison. In the experiments under consideration, Rabbit 1644, possessing a gallbladder infection probably contracted during the immunization with living organisms, disposed rather slowly of the inoculated bacteria. Furthermore, experiments suggest that the blood stream will remain free from viable typhoid bacilli when the spleen has completely disposed of the accumulated microbes. Infarction of the spleen with marked enlargement and a poor exsanguination are some of the factors responsible for the high bacterial count in rabbit 1588.

Certain observations pertaining to the localization of the inoculated bacilli deserve some brief consideration. The liver retains in the first 10 minutes after inoculation from 10 to 20% of the injected organisms. The figures dealing with the lung tissues are also of interest. The lungs of rabbit 1647, immunized with living bacteria, removed from the blood stream about 100 times as many bacilli as the normal, and 10

TABLE 7

The Fate of a Small Dose of B. Typhosus in Rabbits Immunized With Dead and Living Organisms

Tissues	10'			24 Hours			72 Hours		
	Immune Rabbit 1580	Immune Rabbit 1647	Normal Rabbit 1583	Immune Rabbit 1581	Immune Rabbit 1644	Normal Rabbit 1584	Immune Rabbit 1585	Immune Rabbit 1645	Normal Rabbit 1588
Mode of immunization	Heat killed and tri-cresolized (7 injections)	Heat killed and living (7 injections)	....	Heat killed and tri-cresolized (7 injections)	Heat killed and living (7 injections)	....	Heat killed and tri-cresolized (7 injections)	Heat killed and living (7 injections)	....
Agglutination titer....	1:200-400+++	1:6,000+++; 1:20,000+++	<1:10	1:6,000+++	1:8,000-1:10,000+++	<1:10	1:80+++	1:4,000+++ 1:8,000++	<1:10
Left and central liver..	58,000	95,000	30,000	250	4,500	10	5	3	12
Right and central liver	130,000	120,000	6,000	630	19,000	<10	2	1	16
Bile....................	....	0 (0.5 c c)	0 (0.6 c c)	0 (0.1 c c)	12,000,000 (0.9 c c)	0 (0.2 c c)	0 (0.2 c c)	0 (0.8 c c)	0 (0.4 c c)
Gallbladder wall.......	Absent	700	135	1	39,000	0	0	0	0
Spleen.................	40,000	43,000	17,200	180	18	<10	79	19	2,650
Bonemarrow...........	6,800	7,000	2,000	400	6	1	0	0	3
Mesent. lymph nodes..	1	6	0	0 (cocci)	25	2	1	0	0 (coli)
Lungs.................	230	2,400	20	10	1	10	0	0	1
Kidneys...............	0 (20,000 streptococci)	42	11	1	1,130	0	0	0	0
Urine..................	0, 0, 0	0, 0, 0	0, 0, 0	0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0	0, 0
Carotis blood..........	80, 80	400	2,800, 1,300	0, 0, 1, 0, 1	0, 0, 0, 0, 0	0, 0, 0, 0	0 (E:O)	0 (E:O)	0, 0, 1, 0, 0, 0, 0
Heart blood...........	120, 100	900, 100	700, 600	0, 0, 0, 0	0, 0, 0 (E:O)	0, 0, 0, 0 (E:O)	0 (E:O)	0 (E:O)	0, 1, 0, 0, 0
Duodenum.............	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	∞ B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus
Sacculus rotundatus...	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus	Negative for B. typhosus

times as many as rabbit 1580, which was only treated with heat-killed vaccines. As a rule, this behavior of the lung tissue is constant in healthy, immunized rabbits, but may be seriously disturbed by a latent B. bronchisepticus infection, so frequently encountered in animals subjected to bacterial vaccines or their split products. Extensive immunization, however, is conducive to a deposition of bacteria in the pulmonary capillaries. Just why they are removed by the lung of the immunized or sensitized animal we have not as yet determined conclusively, but the recent observations of Coca [21] on sensitized rabbits suggest that an occlusion of the pulmonary vessels is perhaps the most important factor. Most of the investigators dealing with the distribution of bacteria in the normal lung of animals following an intravenous injection conclude that the organisms accumulate in these tissues as a result of mechanical influences, notably the difficulty of dispersing the bacteria in the blood, the small size of the capillaries and the secondary accumulation of leukocytes. According to our observations, neither the dispersion nor the accumulation of leukocytes is notably different in the immunized rabbits, but the lumen of the capillaries is narrowed and assists in the retention of the injected organisms.

In comparing our data with those published by Parker and Franke, we note that as a whole the results of the experiments on immunized and normal rabbits are identical. However, certain figures in their article appear to be incorrect. For example, on page 306 they state that a normal rabbit (1), weighing 1,900 gm., was injected with 39,627,000 typhoid bacilli; the liver retained 15 minutes after the inoculation 270,000 bacteria per 100 mg. of tissue; calculated for the liver weight [22] of this animal, this organ alone would have retained 153,000,000 bacteria. A similar discrepancy exists between the inoculum and the liver count for an immune rabbit 2. The inoculation was stated to be 39 million typhoid bacilli, while based on calculations, the liver removed in 10 minutes 287 million. Throughout this publication the percentage of bacteria retained by the liver tissue varies between 30-60%. These figures are, according to the data furnished by Arima [23] and our own, unquestionably too high, and it appears not unlikely that the counts dealing with the test dose are incorrect. The more recent work of Stone is similarly invalidated by incorrect determinations of the inoculated bacteria. According to her estimates, 460,000 million, or 460 billion were inoculated, and yet at the end of 5 hours the blood stream of one rabbit contained only 30 bacteria per c c. In view of these discrepancies, it is justifiable to assume that in all probability not more than 4,600 million bacteria were actually inoculated. Moreover, in the light of the data of Bail[3] and our own, it appears utterly impossible that the blood and tissues of an immunized rabbit can destroy in 18-24 hours 460 billion typhoid bacilli. The findings of Stone are certainly "paradoxical," to use her own expression, but one is unable to explain the sources of error on account of the sketchy character of the experimental data. It is not unlikely that the method of incomplete tissue cultivation or the preparation of the tissue suspensions is at fault.

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[21] Jour. Immunol., 1919, 4, p. 219.

[22] We have estimated the liver weight of 200 normal rabbits as 1/30 of the body weight.

[23] Arch. f. Hyg., 1911, 73, p. 265.

The results of our experiments with typhoid bacilli intravenously inoculated into normal and immunized rabbits can be briefly summarized:

1. A small number of bacteria is rapidly removed from the blood stream; about 20 to 30% of the inoculum can be found in the liver, a smaller percentage in the spleen, bonemarrow and lungs, and comparatively small numbers in the lymph nodes, kidneys, muscle, etc. The gallbladder bile contains a small number of bacteria, while the gallbladder wall receives, according to its size, a proportional share of the total number of bacteria deposited in the liver. The intraportal injection has no advantage over the intravenous method.

2. Large numbers of typhoid bacilli are less speedily removed from the blood stream, but the distribution in the organs remains the same in the first 4 to 8 hours. The bacteria taken up by the tissues are rapidly reduced in number, particularly in the liver, spleen and lungs. The bonemarrow is less active and not infrequently the typhoid bacilli multiply in these tissues and form foci, which reinfect the blood stream. The gallbladder can become infected either as a result of an immediate extensive elimination of the introduced bacteria, or the continuous discharge of bacilli from liver foci in the hepatic bile, or in consequence of an embolic infarction of the gallbladder capillaries, causing a diphtheric cholecystitis. In the latter instance the cystic bile may not become invaded with typhoid bacilli for 24-48 hours after the intravenous injection.

3. The distribution and destruction of small or large doses of typhoid bacilli is practically the same in the normal as in the immunized rabbit, whether protected by the injection of dead or living organisms.

The only noteworthy difference between the normal and immunized rabbit is found in the lung and spleen; namely, the lung of the immunized rabbit takes up a somewhat greater number of bacteria than that of the normal; and the spleen of the immune sterilizes itself more slowly than that of the normal.

4. Localization in the gallbladder and bile 96-120 hours after the infection, occurs more readily with moderately heavy suspensions prepared from rabbit-blood agar than with those obtained from peptic-digest agar slants.

5. Rabbits which succumb to the typhoid intoxication regularly harbor enormous numbers of typhoid bacilli in the bonemarrow of the long bones; these foci are probably the seedbeds responsible for the continuous invasion of the blood stream and the subsequent overwhelming infection of the liver and spleen.

THE LOCALIZATION AND SUBSEQUENT DESTRUCTION OF PARATYPHOID A BACILLI IN THE TISSUES OF NORMAL AND IMMUNIZED RABBITS INFECTED BY THE INTRAVENOUS ROUTE

It has been stated in the introduction that the paratyphoid A bacillus behaves like the typhoid bacillus in the tissues of rabbits. This conclusion is based on 3 series of experiments—2 conducted with a large number of bacilli (4,500 million and 3,400 million, respectively); and one with a moderately large inoculum (590 million). One experiment is chosen for discussion.

Exper. 8. Five rabbits of 2 litters, about 210 days old, and an average weight of 2,427 gm., were immunized by Bull's method (dead and living bacteria) with a mixture of 3 paratyphoid A strains, isolated from carriers and obtained through the courtesy of Dr. Charles Krumwiede from the New York City Department of Health. Fifteen days after the last injection these rabbits, with an equal number of controls, members of the same litters, were injected intravenously with 3,400 million of a mixture of the paratyphoid A bacilli used for the immunization. After the lapse of 10 minutes, 24, 48, 96 and 144 hours the rabbits were exsanguinated, their organs excised and cultures made by the usual technic.

Two normal and one immunized rabbits showed extensive biliary infections, with correspondingly high counts in the liver tissue. The counts in the splenic tissue and bonemarrow of the normal, as well as the immunized, rabbits were low and practically identical; for example, the spleen cultures showed on the 48th hour for the normal rabbit 79, and the immunized, 70 colonies per 100 mg. of tissue; on the 96th hour for the normal, 106, and 200 for the immunized.

The lung tissue of the immunized rabbit retained 200 times the number of bacteria deposited in the same tissue of the normal. The blood stream of the animals was not sterile before the 96th hour; at the end of 24 hours the blood of the normal animal registered fewer bacteria than the immunized rabbit. Five animals, or 50%, exhibited either renal or urinary bladder infections; the urine of one immune rabbit contained 3,300 paratyphoid bacilli per c c.

The results obtained in these experiments differed in no way from those reported for the typhoid bacillus. The well-known native typhoid immunity, which is apparently responsible for these results in rabbits, is likewise potent for the paratyphoid A. bacillus. The conclusions which have been drawn from the experiments with the typhoid bacillus can be applied unreservedly to the studies conducted with the paratyphoid A bacillus.

THE LOCALIZATION AND SUBSEQUENT DESTRUCTION OF PARATYPHOID B BACILLI (RABBIT AND HUMAN ORIGIN) IN THE TISSUES OF NORMAL AND IMMUNIZED RABBITS INFECTED BY THE INTRAVENOUS ROUTE

It is well known that rabbits may be highly susceptible to paratyphoid B bacilli. One of us (K.F.M.[24]) discussed this subject recently in connection with the description of a spontaneous paratyphoid B epidemic in laboratory rabbits. The isolation of a rabbit-pathogenic organism suggested some experiments along the lines already mentioned for the typhoid and paratyphoid A bacilli. In the course of these tests it was noted that the bactericidal powers of the immunized rabbits excelled those of normal rabbits infected with moderately large doses of virulent animal paratyphoid bacilli. In general the same principle was found to hold true for human paratyphoid B bacilli or B. schottmülleri. One experiment is chosen to discuss this important phenomenon.

Exper. 9. Ten rabbits of 2 litters over 1 year old, of an average weight of 3,080 gm., were used. Five animals were immunized by 3 subcutaneous injections of 0.01, 0.05 and 0.1 c c of a 24-hour old living broth culture of B. aertryckei (strain 1371).[24] Twenty-eight days after the last injection, the serum of these rabbits agglutinated the immunizing strain in dilutions of 1:1000 to 1:10,000, while the nonprotected animals of the same species either failed to clump the antigen, or did so in dilutions not exceeding 1:20. The entire series of rabbits was infected by an intravenous injection of 700 million bacteria of the identical strain, 1371. Exsanguination, excision of the organs and plating were conducted 10 minutes, 4, 24, and 72 hours after the introduction of the infective dose. The remaining normal animal was moribund on the 120th hour and was killed; while cultures were made from the immunized rabbit on the 144th hour. The cultural findings are presented in concrete form in table 8.

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[24] Litch and Meyer, Jour. Infect. Dis., 1920, 28, p. 27.

It is clearly demonstrated in table 8 that the immunized rabbits possess the power to destroy paratyphoid B. bacilli more readily than the nonprotected animals of the same species. These differences are clearly evidenced by the clinical symptoms; the immunized rabbits appear lively and lose little weight, while the normal ones, after an incubation time of at least 24 hours, refuse to eat, exhibit general prostration, labored breathing, severe diarrhea, and loss of from 300 to 500 gm. of body weight. In contrast to typhoid animals in which the evidences of intoxication occur early, these symptoms never develop before the 24th hour. Aside from this striking fundamental difference, there are several other features which distinguish a paratyphoid B infection from the typhoid infection in the rabbit, hence a detailed analysis of the data presented in table 8 is justified.

The ratio of distribution of paratyphoid bacilli in the organs is practically the same as that noted for the typhoid bacillus; again the lung tissues of the immunized animals retain slightly more (2-7 times) organisms than the normal. The predominance of bacteria in the normal spleen is rather constant; this organ not only retains a large number of bacteria, but is frequently unable to dispose of the invaders. In case of recovery from the infection, 15 to 25 days elapse until sterility is established. On the other hand, the spleen of the immunized rabbits becomes free from bacteria in from 6-8 days if less than one billion micro-organisms has been inoculated. Occasional infarction will prolong this period for 10 or more days. These observations furthermore suggest that the persistence of a large number of paratyphoid bacilli in the spleen is in all probability responsible for the organisms in the blood stream. Sterility of the blood in rabbit 1415 was accompanied also by sterility of the spleen and bonemarrow, and was not due to the agglutinin content of the serum. In fact, the importance of the spleen was demonstrated even more distinctly in several additional experiments not reported in this paper. Invariably the number of bacteria found in this organ in normal rabbits shortly before death exceeded that recorded for the liver or bonemarrow. For example, in a moribund rabbit, 1477,

TABLE 8

THE LOCALIZATION AND SUBSEQUENT DESTRUCTION OF PARATYPHOID B. BACILLI (RABBIT ORIGIN) IN THE TISSUES OF NORMAL AND IMMUNIZED RABBITS INFECTED BY THE INTRAVENOUS ROUTE

Tissues	Immune Rabbit 1352 Agglutination 1:8,000		Normal Rabbit 1443 Agglutination 1:60		Immune Rabbit 1353	Normal Rabbit 1445	Immune Rabbit 1416	Normal Rabbit 1447	Immune Rabbit 1444	Normal Rabbit 1444	Immune Rabbit 1415	Normal Rabbit 1446
	10′	4 Hours' Incubation	10′	4 Hours' Incubation	4 Hours in Vivo	4 Hours in Vivo	24 Hours	24 Hours	72 Hours	72 Hours	144 Hours	120 Hours
					1:1,000 3+- 1:4,000 2+		1:8,000 3+		1:6,000 3+		1:8,000–1:10,000	
Left and central liver	111,000	3,400,000	148,000	14,000,000	11,700	118,000	2,720	45,000	4,000 2,850	25,500	230	27,000
Right and central liver	102,000	4,000,000	170,000		11,400	95,000	3,400	41,000	1,500	28,900	570	134,000
Gallbladder wall	156	....	288	....	38	20	13	80	0	10	300,000	18,500,000
Bile	0 (1.5 c c)	....	257 (0.4 c c)	....	44,200	0 (0.45 c c)	0 (0.6 c c)	3,800 (1.1 c c)	0 (0.7 c c)	1 (1.13 c c)	270,000,000 (0.5 c c pus)	18,900,000 per c c
Spleen	6,700	6,200,000	186,000	27,500,000	2,350	23,000	135	219,000	5,400	130,000	1	165,000
Bonemarrow	14,100	1,110,000	22,000	3,600,000	8,000	43,000	450	52,000,000	350	21,300	0	100,000
Mesenteric lymph nodes	42	2,180	38	150,000	12	30,000	62	190	5,600	42,300	36	960
Lungs	12,500	24,000	5,000	200,000	342	312	130 (76)	16,000	152	21,800	0 (sterile)	25,000
Kidneys	230	43,000	1,290	400,000	40	57	4	132,000	3	244,000	430	80 000
Urine	0; 0 per c c	....	0; 0; 0 per c c	....	0; 0; 0 per c c	....	....	....	....	....	1,100 per c c	10,000 per c c
Carotis blood	800; 2,800 per c c	....	5,000; 11,000; 9,500 per c c	....	6; 2; 4 per c c	40; 50; 56; 63 per c c	11; 15; 8 per c c	3,500; 4,160; 3,400 per c c	3 per c c	97, 142 per c c	0 per c c	....
Clotted blood	220	136	2,300	600,000	....	....	....	....	....	....	....	....
Heart blood	....	....	....	....	....	....	13 per c c	4,090 per c c	6, 70 per c c	690, 480, 1,200 per c c	0 per c c	600; 2,100; 680 per c c
Contents of:												
Duodenum	Sterile	....	1 B. paratyphoid colony	....	Sterile on green plate	Sterile	Neg. for B. paratyphoid B	Neg. for B. paratyphoid B	Neg. for B. paratyphoid B	∞ B. paratyphoid B	∞ B. paratyphoid B	∞ B. paratyphoid B
Jejunum	....	....	....	....	Sterile on green plate	Sterile	Neg. for B. paratyphoid B	1 colony B. paratyphoid B	Neg. for B. paratyphoid B	∞ B. paratyphoid B	6 B. paratyphoid B	....
Ileum	....	....	....	....	....	....	Neg. for B. paratyphoid B	....	Neg. for B. paratyphoid B	....	....	....
Sacculus rotundatus	....	....	....	....	1,000 B. paratyphoid B	....	25 B. paratyphoid B	10 B. paratyphoid B	Neg. for B. paratyphoid B	∞ B. paratyphoid B	∞ B. paratyphoid B	....
Appendix	....	....	....	....	33 B. paratyphoid B	....	37 B. paratyphoid B	200 B. paratyphoid B	Neg. for B. paratyphoid B	∞ B. paratyphoid B	250 B. paratyphoid B	....

exsanguinated on the 40th hour, the spleen contained 88 million paratyphoid bacilli per 100 mg., the bonemarrow 22 million, the liver 11 million, and the blood 75,000 bacteria. The activities of the spleen are further indicated by the anatomic response of the organ; a spleen tumor, together with an increased weight, is rather constant. The average splenic weight for the immunized animal was 2.61 gm., and for the normal, 2.15 gm., in certain individual cases a weight of 4.25 and 3.36 gm., respectively, was recorded. Compared with the normal weight and estimated per 1,000 gm. of bodyweight, it was calculated that the increase in weight is more marked in the immunized than in normal rabbits, which in turn suggests an increase in cellular elements. It has been shown by F. A. Evans[25] that the acute splenic tumor of rabbits infected with paratyphoid-like bacteria (B. enteritidis) is characterized by hyperplasia of the phagocytic reticulo-endothelial macrophages, and by a decrease in the number of the other cells of the pulp. The latter condition is probably the result of a toxic inhibition of the leukopoietic function and perhaps a stimulation of the functional reticulo-endothelial cells. The lymphatic nodes also exhibit, in certain rabbits, remarkable affinity for the paratyphoid bacilli, the significance of which fact is not quite clear, but deserves further elucidation. In the first 24 hours the differences between immunized and normal rabbits are fairly uniform; the blood, liver and spleen of the latter contain 10 times as many living organisms as the former. For the lung tissue this contrast is not so clearly demonstrated on account of the initial high count. After 24 hours the actual number of bacteria demonstrable in the organs of the individual rabbits may fluctuate considerably and less uniform counts are obtainable. The focal necroses and embolic processes (gallbladder), which develop quite frequently in immunized animals, and the variations in the individual resistance even in litters, are conducive to these differences. Focal necroses in the bonemarrow of rabbit 1447 and bacterial emboli indicated by hemorrhages in the kidney cortex of the same rabbit, as well as in rabbit 1444 and rabbit 1446, are probably responsible for the high bacterial counts recorded .

Two normal and 2 immunized rabbits developed biliary infections. The finding of paratyphoid bacilli in the bile of 2 animals in the first 24 hours is probably the result of an elimination of these organisms by way of the biliary passages, and not the sequel of a gallbladder wall infection, as noted for rabbit 1415 and rabbit 1446. This phase of the

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[25] Bull. Johns Hopkins Hosp., 1916, 27, p. 356.

problem has already been discussed by Litch and Meyer in a recent paper on rabbit typhoid (100). These observations likewise confirm the statement that rabbits intravenously inoculated may fail to register bacteria in the bile 24-72 hours after an intravenous injection, and yet may develop a chronic cholecystitis as a result of bacterial embolism of the gallbladder wall. Every nonimmunized rabbit which succumbed to the intravenous infection on the 4th-11th day exhibited a diphtheric, necrotized cholecystitis, although repeated observations in supplementary tests failed to indicate that paratyphoid bacilli are discharged through the bile stream in the first 30 minutes following an intravenous injection of a number of bacteria large enough to insure a general infection and death in from 3 to 5 days. After a lapse of 24 hours bacteria are undoubtedly eliminated by the bile, as is suggested by the positive cultures obtained from the duodenal contents of rabbit 1444, while the gallbladder is sterile, or nearly so, and the wall of this viscus contains a few bacteria corresponding entirely to the degree of the general blood infection. In this connection another difference between the typhoid and paratyphoid infection of the rabbit is noted. The contents of the ileum, sacculus rotundatus and appendix of the typhoid rabbits never demonstrated specific bacteria without positive findings in the duodenum and jejunum, while in several animals (rabbits 1353, 1416 and 1447) of exper. 8, paratyphoid bacilli could be demonstrated in the above mentioned portions of the intestinal tract without receiving their seedings from the upper segments or the bile. It is not unlikely that the constant involvement of the lymphatic structures of the intestines of paratyphoid rabbits leads to a discharge of specific bacteria into the intestinal content, producing the positive cultures recorded. Besredka [26] has recently called attention to this localization of Shiga dysentery and paratyphoid bacilli in the large intestines of rabbits and, based on these observations, he has developed an attractive theory on the local immunity of the intestinal mucosa to specific bacterial invasion. In connection with the discussion of our feeding experiments with organisms of the typhoid-paratyphoid group, this subject will be considered.

It has already been stated that the observations recorded in the foregoing were confirmed by several experiments with human paratyphoid strains. As distinct serologic differences have been demonstrated between these strains and true B. schottmülleri by numerous independent workers, and as it is as yet undecided whether the paratyphoid infection in man is identical with "rabbit typhoid," we repeated our immunity tests with several strains of B. schottmülleri isolated from typical human cases and sent to us by Mr. Harry Weiss of the Department of Bacteriology, Columbia University. Using a test inoculum (1-2 billion), which caused death after 6 to 8 days in the nonimmunized rabbits, the results were practically identical with those reported for the animal strains. An ability to destroy paratyphoid B bacilli more quickly is also evidenced in the immunized animals when a small number (300 million) of bacilli is intravenously inoculated; in fact, this phenomenon is particularly striking for the blood. To dispose of a number of organisms which can be destroyed by a normal animal in 5-6 days, an immune rabbit requires less than 24 hours. But in no instance was a cholecystitis produced: the number of bacilli injected was not sufficient even to cause an early discharge in the hepatic duct bile.

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[26] Ann. de l'Inst. Pasteur, 1919, 33, pp. 301 and 557.

In conclusion, it may be stated that the mechanism of destruction of the paratyphoid B bacilli when intravenously inoculated into immunized and normal rabbits differs from that constantly observed for the typhoid bacillus. This applies whether one uses B. aertryckei of rabbit origin or B. schottmülleri. Paratyphoid B. bacilli disappear more quickly from the tissues of the immunized than of the normal animals. It appears of interest in this connection to inquire into the nature of the factors responsible for this immunity, whether resident in the blood or tissues.

ATTEMPTS TO DETERMINE BY IN VITRO EXPERIMENTS THE FACTORS RESPONSIBLE FOR THE DESTRUCTION OF TYPHOID OR PARATYPHOID B. BACILLI IN THE RABBIT BODY

The original conception of Pfeiffer and his pupils, based on experiments primarily conducted on guinea-pigs, attributed the resistance to typhoid fever to bacteriolytic properties of the blood and lymph stream. This interpretation of the acquired immunity was challenged by Metchnikoff and his school, who believed that the leukocytes were mainly responsible for the destruction of the typhoid bacillus in the blood stream or the tissues of the immune animal or man. Based on the well-known experiments of Wassermann and Citron,[27] which demonstrated that antibodies may be produced by any cell or cell complex brought in contact with pathogenic bacteria, the theory of the local tissue immunity was proposed, but attracted little attention. However, in recent years the conception that in man the resistance to typhoid fever fever acquired during recovery from this disease is cellular and not humoral, has gained some encouraging experimental support. In this connection the studies of Wassermann and Sommerfeld [28] on mice, of Besredka [29] on rabbits, and of Liebermann and Acel [30] on guinea pigs, and the critical summary of Bessau,[31] which bridges the humoral and cellular theory in an attractive manner, should be mentioned. These studies are of great value from an experimental standpoint, but as we are unable to state whether the immunity mechanism of the guinea-pig, rabbit or mouse is identical with that in man, far reaching conclusions and analogy deductions are unquestionably premature and unjustified.

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[27] Deutsch. med. Wchnschr., 1905, 31, p. 573.

It is interesting to read in many publications dealing with the behavior of the typhoid bacillus in experimental animals, the statements that it is impossible to provoke in the usual laboratory animals, including the chimpanzees, a disease similar to typhoid fever in man, and that for these reasons the various factors and processes accompanying a human infection cannot be reproduced, nor can they be followed in the course of their intricate development. Yet the same writers usually untrained in comparative pathology, do not hesitate to regard certain facts elucidated, for example, on rabbits as indicative of a close analogy to the infection produced by the typhoid bacillus in man. It appeared to us that progress in the understanding of the mechanism of typhoid resistance could perhaps be made by analyzing our knowledge concerning the nature of the factors responsible for the native and acquired immunity of the rabbit against the typhoid or paratyphoid B bacillus. The in vivo experiments reported in the preceding paragraphs confirmed the observations of Bull, Arima, and Parker and Franke. Typhoid bacilli intravenously injected into rabbits are primarily destroyed in the liver, spleen, bonemarrow and lung. Even in the immunized rabbit the progress of the destruction of the deposited bacilli differs in no respect from that established for the normal animal. The test-tube experiments of Buxton,[32] Bull,[33] Teague and McWilliams,[34] and Stone showed, however, that the blood serum, plasma or defibrinated blood of immunized rabbits is nonbactericidal for the typhoid bacillus, while normal serum can destroy in 5-24 hours from 100,000-5,000,000 organisms. As numerous experiments conducted by us with the blood, its fluid and cellular elements produced results similar to those reported by these workers, it is considered unnecessary to present our additional data. It therefore may be surmised that the destruction of typhoid bacilli in the normal or unimmunized rabbits either cannot primarily be the result of bacteriolytic serum or lymph substances, or the in vitro experiments fail to reproduce the conditions as they exist in the rabbit body, a view held by Bail in 1905. The latter conception is apparently supported by some tests which we conducted with the serum of rabbits immunized against paratyphoid B bacilli.

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[28] Med. Klin., 1915, 11, p. 1307.

[29] Ann. de l'Inst. Pasteur, 1919, 33, pp. 301 and 557.

[30] Deutsch. med. Wchnschr., 1917, 43, p. 867; 1918, 44, p. 313; Biochem. Ztschr., 1918, 91, p. 46.

[31] Deutsch. med. Wchnschr., 1916, 42, p. 499.

[32] Jour. Med. Research, 1905, 13, pp. 305 & 431.

[33] Jour. Exper. Med., 1914, 20, p. 237.

[34] Jour. Immunology, 1917, 2, pp. 167, 185 and 375.

Exper. 10. Action of the Serum and Whole Blood of Normal and Immunized Rabbits in Vitro on Paratyphoid B Bacilli (B. Aertryckei).—The defibrinated blood or the fresh serum of normal healthy rabbits, or of those immunized by 3 subcutaneous injections with small doses of living paratyphoid B bacilli were tested for agglutinating value and for bactericidal power by means of the Buxton[32] or Teague and McWilliams[34] technic.

Thermolabile agglutinins for paratyphoid B bacilli in dilutions varying from 1:2-1:10 were demonstrated in the 4 rabbits tested for this purpose. The immune agglutinins of the protected rabbits reached frequently a titer of 1:10,000 and over.

One c c of fresh serum obtained from the heart blood of the normal and immunized rabbits was absolutely devoid of bactericidal properties for the B. aertryckei (rabbit paratyphoid B). Not even 5-15 organisms were destroyed in 24 hours. These serums in equal amounts were readily able to kill from 40,000-500,000 typhoid bacilli after an exposure of 24 hours. Similiar results were obtained with defibrinated blood. Human paratyphoid B bacilli reacted more irregularly; some rabbit serums would destroy from 100-1,000 bacteria. The human strains employed were about 2 years old; they had lost their invasive properties for the rabbit and in turn became more susceptible to the bactericidal powers of the blood.

These observations are not new. Citron,[35] Toepfer and Jaffe,[36] Neufeld and Hüne,[37] Werbitzki,[38] and recently Teague and McWilliams[34] have noted that the normal or immunized rabbit serum may possess no bacteriolytic substances while tropins can be demonstrated. In fact, Citron[35] and Tenbroeck[39] concluded from numerous experiments with the B. suipestifer that the presence or absence of serum antibodies does not permit conclusions relative to the degree of resistance of the rabbit against a subsequent infection.

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[35] Ztschr. f. Hyg. u. Infektionskrankh., 1906, 53, p. 515.

[36] Ibid., 1905, 52, p. 407.

[37] Arb. a. d. k. Gsndhsamte, 1907, 25, p. 164.

[38] Arch. f. Hyg., 1909, 70, p. 271.

[39] Jour. Exper. Med., 1917, 26, p. 441

The evidence thus far at our disposal suggests, therefore, that the destruction of typhoid as well as paratyphoid bacilli in the immunized rabbit cannot be explained by in vitro experiments with the whole blood or serum. Impressed by the conception of an histogenous immunity, so ably demonstrated for streptococcus infections in the cat, rabbit and guinea-pig (Hopkins and Parker, and Nagao),[40] and for staphylococcus infection in the dog (Bartlett, Ozaki [41] and Ito [42]) we were led to conduct numerous in vitro experiments with the tissues of infected normal and immunized rabbits. Typhoid and paratyphoid bacilli were used. One experiment is chosen to demonstrate the results obtained.

Exper. 11. Bactericidal Effect of the Tissues of the Normal and Immunized Rabbit Against B. Typhosus in Vitro and Vivo.—Two normal rabbits and 2 members of the same litter, which had been immunized with living typhoid bacilli according to Bull's method, were inoculated intravenously with 2,250,000 typhoid bacilli strain K. per 100 gm. of body weight. The average weight of the 4 rabbits was 2,860 gm. At stated intervals the animals were bled to death under ether. The organs were removed and distributed in sterile test tubes, which were placed in a water bath at 37 C. for from 2-5 hours. At the end of this time the pieces were weighed, ground and plated, as stated in previous experiments. The bactericidal effect of the leukocytes was tested by using clotted blood. The method given by Hopkins and Parker was followed. The results are summarized in table 9.

It will be noted from table 9 that at the end of the first 2 hours of incubation, the lung tissues of both the normal and immunized rabbits exerted in vitro a distinct bactericidal effect on the typhoid bacilli. There was a similar reduction in the spleen and bonemarrow, while in the liver, mesenteric lymph nodes, and kidneys the organisms multiplied moderately. Destruction by the excised tissue took place only during the first 2 or 3 hours of incubation, after which, owing probably to death of the cells, the organisms multiplied. On the other hand, in vivo the destruction progressed continuously and primarily marked in the lung and liver. At the end of the first 12 hours the lung tissue contained from $\frac{1}{30}$-$\frac{1}{50}$, and the liver from $\frac{1}{40}$-$\frac{1}{80}$ of the original number of typhoid bacilli deposited in these tissues. The spleen was less active, while the bonemarrow showed a distinct increase. The data pertaining to the clotted blood suggested that the leukocytes had exerted their supposed bactericidal effect. It should be stated, however, that the method of obtaining these results was not very accurate. In control

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[40] Jour. Infect. Dis., 1920, 27, p. 327.

[41] Jour. Med. Research, 1916, 35, p. 465.

[42] Ibid., 1917, 37, p. 189.

TABLE 9

BACTERICIDAL EFFECT OF THE TISSUES OF THE NORMAL AND IMMUNIZED RABBIT AGAINST B. TYPHOSUS IN VITRO AND VIVO

Tissues	Rabbit 1287; Immunized and Agglutinated; 1:100,000; 6.3 Billion Injected			Rabbit 1287 (Normal 1:20+); 5.7 Billion Injected			Rabbit 1288 (Immune) (5,040,000,000)	Rabbit 1288B. (Normal) (6.3 Billion)
	10′ after Infection, Plated Immediately*	Plated after 2 Hours' Incubation*	Plated after 5 Hours' Incubation*	10′ After Injection, Plated Immediately*	Plated After 2 Hours' Incubation*	Plated After 5 Hours' Incubation*	Exsanguinated and Plated 12 Hours after Injection*	Exsanguinated and Plated 12 Hours after Injection*
Left and central liver	300,000	980,000	2,650,000	900,000	810,000	3,000,000	8,000	5,000
Right and central liver	....	....	....	....	....	....	4,800	6,800
Spleen	130,000	110,000	2,800,000	550,000	460,000	7,200,000	59,000	26,000
Bonemarrow	62,000	25,000	800,000	21,000	15,000	22,000	225,000	88,000
Mesenteric lymph nodes	1,070	1,240	15,000	200	100	2,600	84	28
Lungs	70,000	30,000	700,000	20,000	10,000	60,000	1,200	740
Kidneys	1,600	3,600	40,000	2,200	1,800	27,000	112	64
Urine	0	....	....	0 (enriched +)	....	....	0	0
Femoral muscle	10	6	....	1	1	....	0	2
Gallbladder wall	5,100	....	....	28,000	....	....	10	6
Bile	1 (0.4 c c)	....	....	0 (0.7 c c, also E)	....	....	1 (0.8 c c)	0 (1.8 c c)
Carotis blood	1,200 per c c	....	....	5,500 per c c	....	....	1,500; 2,000; 1,000 per c c	840; 760 per c c
Right ventricle	900 per c c	....	....	....	....	....	1,860 per c c	640 per c c
Left ventricle	300 per c c	....	....	1,400 per c c	....	....		
1 c c of blood clotted immediately	320	50	36	1,750	140	230; 300	....	....
Duodenal contents	13 colonies B. typhosus	....	....	Negative for B. typhosus	....	....	Negative for B. typhosus	Negative for B. typhosus

* Number of colonies per 100 mg.

experiments a moderate multiplication took place, even in the first 3 hours of incubation. The solution of the question dealing with the typholytic effect of rabbits' leukocytes, in the light of the extensive investigations of Bail, Schneider and others, was sufficiently important to warrant a more detailed inquiry than we were able to conduct, at least for the present.

The degree of the destructive action of lung tissue in vitro is not constant. In some experiments it is decidedly more marked than in the one just reported. This statement is supported by the figures in table 10.

TABLE 10

COLONIES PER 100 MG. OF LUNG TISSUE

	Rabbit 1265B Typhoid	Rabbit 1265 Typhoid	Rabbit 1142 Typhoid Immunized	Rabbit 1412 Paratyphoid B Immunized	Rabbit 1481 Paratyphoid B Normal
Plated immediately.................	14,400	110,000	226,000	20,800	3,800
Plated after 4 hours' incubation....	4,900	1,380	26,000	159,000	100,000

Observations made with paratyphoid B infected rabbits demonstrated a complete absence of bactericidal powers of the organs on incubation in vitro. Even the lung tissues were inactive. These facts are illustrated by the data presented in tables 8 and 10.

The results of the various experiments apparently established the fact that the organs of rabbits, with the exception perhaps of the lung, are unable to destroy typhoid or paratyphoid bacilli in vitro. The liver particularly, which in vivo is especially endowed to dispose of enormous numbers of bacteria, is inactive in the test tube. An explanation of this phenomenon is not possible, but it is certain that the bactericidal power of the rabbit organs is not only closely connected with a function of living protoplasm but is probably the result of an interaction between the blood and lymph substances and the tissue elements. Whether the polymorphonuclear leukocytes perform this function either alone or in conjunction with endothelial living cells and endothelial leukocytes (N. C. Foot),[43] together with certain blood plasma elements has not been determined. The histologic studies of Kyes,[44] of Hopkins and Parker,[45] of Bartlett, Ozaki and Ito [41] and of Nagao [40] with gram positive organisms, such as pneumococci, streptococci, staphylococci, respectively, and the observations of Schneider [46] with typhoid bacilli, indicate that both fixed and wandering phagocytes (macrophages) have the ability to engulf, as well as to digest, micro-organisms. The histologic studies of Mallory [47] and Gräff [48] established conclusively the fact that the typhoid bacillus produced in the reticulo-endothelial tissues of the lymph nodes, spleen, bonemarrow, liver, etc., a specific proliferation of the tissue histiocytes (Aschoff and Kiyono [49]). A casual study of the sections derived from the animals used in this report clearly demonstrated the remarkable phagocytic action of the endothelial cells of the liver and spleen and confirmed the well-known active function of the polymorphonuclear leukocytes. Several attempts to study the localization of the typhoid bacilli in their relation to the various cells have not been regularly successful. Even the best staining method (thionine, method of Zieler) produced indistinct and misleading pictures. Occasionally Kupffer's cells were found to be packed with bacilli when rabbits killed on the 6th hour were studied. Attention was therefore given to the general appearance of the various cells. Proliferation of the vascular endothelium in the spleen, liver, lung and lymph nodes of rabbits infected with B. aertryckei has already been mentioned by one of us in a previous communication, and it was pointed out on this occasion that these vascular reactions suggested a distinctive cellular mechanism of defense. The changes which can be noted in the sections were remarkably similar to those recently described by Nagao [40] for the streptococcus, and a detailed account of the data is contemplated in a paper of this series.

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[43] Ibid., 1919, 40, p. 353.

[44] Jour. Infect. Dis., 1916, 18, p. 277.

[45] Jour. Exper. Med., 1918, 27, p. 1.

The functions of the polymorphonuclear leukocytes as phagocytes, or as sources of typholytic substances, or as cells endowed with specific immunizing properties (Bachman [50]) cannot be denied since these properties can be demonstrated in vitro and vivo. The observations of Bull [7] on phagocytosis in vivo have been confirmed repeatedly. In thionine-stained smears made from the lung tissue, in preference to the liver and spleen pulp, of rabbits which had been inoculated with moderately large doses of B. typhosus and killed 10-60 minutes after the injection, we were able to count numerous polymorphonuclear leukocytes retaining intact and phagolyzed rods. In the paratyphoid B infected animals this phagocytosis occurred to a noticeable degree in the immunized, and to a lesser degree, in the normal rabbit. Whether this phagocytosis, however, only initiates the destructive mechanism cannot be investigated with certainty in vivo. We gained the impression that it was more or less a temporary process, but the subject will be considered more carefully in the next paper in connection with the analysis of the mechanism leading to the elimination of bacilli in the bile. Opportunity will be afforded to discuss the changes in the distribution of the polymorphonuclear leukocytes, which follow an intravenous injection in the first 5-120 minutes. Since the bacilli are taken up by the leukocytes it appears not at all unlikely that they are digested by a leuko-enzyme; this conception is supported by the demonstration of an ereptic enzyme in the rabbit's leukocyte by Parker and Franke.[51]

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[46] Arch. f. Hyg., 1909, 70, p. 41.

[47] Jour. Exper. Med., 1898, 3, p. 611.

[48] Deutsch. Arch. f. klin. Med., 1918, 125, p. 352; 126, p. 1.

[49] Folia haematol., 1913, 15, p. 383.

[50] Rev. de la Assoc. méd., Argentina, 1918, 29, p. 549; Rev. méd. del Rosario, 1919, 9, p. 1; Prensa méd., Argentina, 1919, 5, p. 253.

We desire to recall the observations of R. Schneider,[52] who showed by a set of ingenious experiments the active secretion of thermostabile bactericidal substances by leukocytes suspended in inactivated serum. This vital secretory function of the leukocytes is readily paralyzed by asphyxia of the cells with CO_2. The in vitro experiments conducted with rabbits' tissues by us and others have ignored this important fact. Is it not possible that the CO_2 content of the liver, spleen and bone-marrow in vitro inhibits the function of the leukocytes? It is, however, possible that the liver and spleen contained few leukocytes at the time the rabbit was exsanguinated, and the lung retaining the majority of these cells was particularly endowed to exhibit the described bactericidal properties. An investigation, with these conceptions in mind, is earnestly needed, as positive results would materially help to elucidate the many unexplainable impressions obtained through test-tube experiments. Attention should also be paid to the recent studies of Bachman [50] on a specific substance demonstrable in the polymorphonuclear leukocytes of immunized animals. This may, for example, prove that the leukocytes of rabbits immunized against paratyphosus B display a new property even in vitro, namely, a specific phagocytosis.

From this discussion it is quite evident that bactericidal substances directed against the typhoid and paratyphoid B bacilli are primarily of cellular origin, and are probably produced when needed at the places of bacterial invasion. In reviewing these facts one is once more reminded of the early studies of Wassermann and Citron [53] on local tissue immunity. According to the observations of these workers, it is proved that the tissues which come in intimate contact with the typhoid bacilli are capable of reacting locally by the production of specific bactericidal substances. For example, the cells lining the pleural cavity cannot only bind the specific antigen, but respond promptly by a production of bactericidal substances. Besredka [54] expresses a somewhat similar idea in his explanation of the intestinal immunity against paratyphoid-dysentery bacilli in the rabbit: "l'immunité naturelle vis à vis du virus typho-paratyphique, l'immunité acquise artificiellement repose également sur l'immunité de la paroi intestinale, ce qui veut dire qu'elle est locale."

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[51] Jour. Med. Research, 1919, 39, p. 301.

[52] Arch. f. Hyg., 1909, 70, p. 41.

[53] Deutsch. med. Wchnschr., 1905, 31, p. 573.

Some observations on the spleen discussed in the preceding paragraphs suggest that such a mobilization of bactericidal substances takes place in the organ itself. Ozaki [55] demonstrated that the accumulation of bacteria in the spleen was principally dependent on the vital activity of the cells, and Morris and Bullock [56] inferred from their extensive experiments that the spleen normally aids tremendously in resisting infectious processes in rats. These facts directed our attention to the spleen. It was constantly noted that in animals of the same litter the disappearance of the deposited typhoid bacilli was slow in the first 1-2 hours after the inoculation, irrespective of the marked local hyperleukocytosis and in contrast to the great reduction of their number in the liver and kidney. For example, in the first hour after the introduction of the infective dose, the spleen contained $1/3$ of the originally deposited bacilli, while at the end of the fourth hour, $1/12$; at the fifth, $1/25$; at the eighth, $1/80$; and at the end of the 24th hour, $1/250$ to $1/400$ of the original number was recordable in the spleen by the plating method. The figures dealing with the liver of the same animal were at the 8th hour, $1/40$; and at the end of the 24th hour, $1/100$ of the original number could be noted. These findings are subject to several explanations. From the recent studies of Teague and McWilliams,[57] it would seem that bacteriolytic substances could readily pass from the blood capillaries to the lymph, and by this route to the spleen and liver, which had been heavily invaded by typhoid bacilli. This hypothesis is applicable to an explanation of the destruction of typhoid bacilli in normal rabbits, but as these forces are not demonstrable in the typhoid and paratyphoid immunized rabbits, the conception of a cellular or a histohumoral immunity mechanism has far more justification. No doubt the suggestions of Manwaring and Coe,[58] dealing with the so-called endothelial opsonins, and the histologic studies of Nagao,[40] also deserve some attention in future experimental investigations.

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[54] Bull. de l'Inst. Pasteur, 1920, 18, p. 129.

[55] Jour. Med. Research, 1917, 36, p. 413; 37, p. 247.

[56] Ann. Surg., 1919, 70, p. 513.

[57] Jour. Immunology, 1917, 2, p. 375.

Additional observations illustrating phases of local immunity in the liver of immunized rabbits and guinea-pigs will be presented in paper 5. In connection with these experiments, it was repeatedly noted that the liver of rabbits which rapidly destroyed enormous numbers of typhoid bacilli were exceedingly voluminous, soft and heavy. For example, rabbits weighing from 2,000 to 2,200 gm. possessed, 4 to 8 hours after the inoculation, livers weighing 122 to 128 gm. These organs instead of representing $\frac{1}{30}$ had increased to $\frac{1}{16}$ of the body weight. A marked edematous infiltration characterized such livers histologically, and the condition must be interpreted as lymphostasis. We are not prepared to draw definite conclusions from this observation, but the well-known fact is recalled that rabbits' lymph obtained by stasis is strongly bacteriolytic for typhoid bacilli, and that the typholytic substances of such body fluids are of leukocytic origin (Schneider [52] and others).

The ability of excised pieces of lung tissue to destroy in vitro a large number of typhoid bacilli is apparently a fairly common function, having been demonstrated for streptococci and staphylococci. Moreover, it is certain that the perfused organ, freed from blood and, to a certain degree, of lymph, is capable of this action. Intercurrent infections, such as bronchopneumonia caused by B. bronchisepticus, seriously impair this destructive property. Moreover, extracts of the lung tissue are inactive. Whether some of the bactericidal substances (polymorphonuclear leukocytes) are preformed in the native immune rabbit, or whether they are mobilized locally, as in the paratyphoid B susceptible animal, is as yet unknown. According to Deutsch [59] the lung tissue of normal guinea-pigs always contains more agglutinins than the serum, while Briscoe [60] claims that phagocytosis by the pulmonary macrophages is more active and rapid when the animal has been previously immunized. The evidence thus far collected experimentally indicates, however, that the bactericidal substances are dependent on the action of the living cells. The entire mechanism of immunity in the lung is probably strictly cellular in origin and action, and is not influenced by a process of immunization. This definitely demonstrable histogenous bactericidal property, which is inherent in the lung is, however, of subordinate influence on the general mechanism of typhoid immunity in the rabbit. A careful experimental analysis of the pulmonary bactericidal powers will no doubt materially assist in disproving that humoral factors alone govern defensive and destructive immunity in typhoid fever and allied diseases.

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[58] Ibid., 1916, 1, p. 401.

[59] Ann. de l'Inst. Pasteur, 1899, 13, p. 689.

[60] Jour. Path. & Bacteriol., 1907-08, 12, p. 66.

THE NATURE OF IMMUNITY IN THE RABBIT AGAINST THE TYPHOID AND PARATYPHOID B BACILLUS

The studies of Bail,[61] of Hiss[62] and of Parker and Franke[51] indicate that the disease artificially produced in rabbits by the intravenous injection of typhoid bacilli is rather a toxemia than an infection ("ein Giftod"—Bail; "acute intoxication"—Hiss). Parker[63] has recently proved that the livers of very sick rabbits contain specific saline-extractable poisons. These toxins are of cellular origin and are the product of an interaction between the living cells of the liver, of other organs and the killed and disintegrated typhoid bacteria. Intoxication and death result primarily in those rabbits that possess a marked power to destroy bacteria in the liver and spleen. The susceptibility is therefore due to the bactericidal properties of its blood and tissues. Resistance to this intoxication is produced by active immunization with living typhoid bacilli, while typhoid immune serums failed to protect against the liver poison. We were able to confirm fully the observations of these workers. Rabbits capable of destroying in 1 c c of serum from 1,000,000 to 5,000,000 typhoid bacilli readily succumbed to the intoxication in from 5 to 48 hours, when injected with from 5 to 10 billion typhoid bacilli; and extractable liver poisons could be demonstrated in every instance. On the other hand, rabbits lacking typholytic substances in the blood or serum, and particularly immunized animals, would show symptoms of intoxication, although liver poison could not be found. The nonsusceptibility is either due to an absence of lytic antibodies or the result of a tolerance to the liver poison acquired in the course of the process of immunization. On the other hand, rabbits injected with paratyphoid B bacilli in moderate doses (less than 1,000 million) fail to show signs of intoxication for at least 48 hours; at the time of death their blood and organs contain enormous numbers of bacteria, but no poisons can be extracted from the liver. Paratyphoid infections in the susceptible rabbit exhibit all the signs of a septicemia. Instead of a progressive destruction of organisms in the blood and organs, as is shown for the typhoid rabbit, a progressive increase takes place. Resistance to this group of bacteria is developed by factors which prevent their multiplication. In vitro experiments indicate that this property is not in the blood serum, but confined to the living cells, and is only acquired by active immunization with living paratyphoid bacilli, a fact demonstrated by Citron[35] and TenBroeck.[39] The invasive B. aertryckei or the B. suipestifer produces a septicemia in the rabbit, while the typhoid bacillus causes a toxemia. It should, however, be borne in mind that only very large doses of living typhoid bacilli are capable of causing this intoxication in susceptible rabbits, while even small doses of paratyphoid B bacilli produce a fatal septicemia. Moreover, the defensive mechanism against the intoxication, which saves the life of the animal, is called on during the first 10 hours after the injection of the typhoid bacillus, while in the paratyphoid infection a similar mechanism may play a rôle only toward the end of the disease. It is, however, well known that typhoid rabbits may die from a moderately large initial injection on the 3rd to the 10th day. In these instances we have failed to find extractable liver poisons, but have demonstrated enormous numbers of bacteria in the bonemarrow, a moderate number in the spleen and liver, and few or no organisms in the blood. For illustration of this point the reader is referred to rabbit 1686 in table 6. Our observations unquestionably corroborate the conclusions of Parker and Franke that in rabbits which are slowly dying of typhoid the bone-marrow may constitute a focus of infection from which the organisms are continually swept into the blood stream and from there taken up by the organs. The actual mode of this localization, whether in the epiphysis marrow or elsewhere, is of great interest but by no means clearly understood (I. Koch[64]). Comparative leukocyte counts by Studer,[65] C. W. Wells[66] and our own indicate that the individual response of the bone-marrow of rabbits, following the injection of typhoid bacilli may vary considerably. In a certain percentage of animals serious disturbances in the leukocytogenic functions follow the injection of typhoid, paratyphoid and coli bacilli, which in turn lead to an exhaustion of the leukocytes and to a breakdown of the defensive mechanism of the body. Future studies should attempt to correlate these observations. It is, however, not unlikely that the numerous infarcts of the liver and spleen going on to necroses as the result of typhoid toxemia constitute also seed beds for the invasion of the blood stream. These toxic necroses lack proper lymph circulation, according to Gräff [48] and favor, as has been demonstrated by Benian,[67] the development of immune body-fast, parasitic typhoid bacilli. Furthermore, the protection of pathogenic organisms by living, endothelial cells, shown by Rous and Jones,[68] must be kept in mind. Unhindered development of the bacteria in the gallbladder constitutes, in our experience, only in exceptional instances a focus responsible for the infection of the blood stream, as will be demonstrated in paper 5, on the mechanism of gallbladder infection.

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[61] Arch. f. Hyg., 1905, 52, p. 272; Wien. klin. Wchnschr., 1907, 20, p. 275.

[62] Jour. Med. Research, 1913, 28, p. 389.

[63] Jour. Exper. Med., 1918, 28, p. 571.

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[64] Ztschr. f. Hyg. u. Infektionskrankh., 1911, 69, p. 436.

[65] Thesis, Univ. of Zürich, 1903.

[66] Jour. Infect. Dis., 1917, 20, p. 219; 1918, 22, p. 502.

Continuous invasion of the blood stream and removal of the organisms by the viscera is accompanied, in the first place, by an accumulation of typhoid bacilli in the liver and in the spleen. In these tissues the main destruction takes place; the liberation of toxic albumoses by disintegration, or the production of toxic substances by some form of proteolysis, unquestionably produces a profound injury, which can be seen macroscopically. The inability to demonstrate poison in the liver is probably due to the fact that its liberation is gradual and in amounts which do not exceed the detoxicating threshold of the organs. Yet this detoxicating function, which probably is present in other organs than the liver, continues as long as bacteria are swept into the blood from the various foci mentioned; but the prolonged production of toxins may seriously damage the metabolic function of the organs, causing cachexia and the death of the animal. Immunized rabbits, which succumb to very large injections of typhoid bacilli, present the same cultural findings (of a low blood and a high bonemarrow count). It appears, therefore, not unlikely that aside from the tolerance against specific poisons, immunity against the typhoid bacillus in rabbits is represented by their ability to prevent the formation of bonemarrow and visceral foci.

In rabbit paratyphoid the distribution of bacteria in the blood and organs is quite uniform shortly before death on the 6th or 7th day, irrespective of the original route of infection. The blood contains large numbers of bacteria per c c, and the tissues a proportional share, according to size. The septicemia is probably the consequence of the washing out of organisms from the foci of active multiplication. The enlarged spleen, in case the gallbladder is not infected, is the chief source; while the bonemarrow, liver and lungs are less important. Liver poisons cannot be demonstrated for the reasons above outlined, but the anatomic lesions and the clinical symptoms suggest that the death of the animal is probably the result of an intoxication. Immunity against a paratyphoid B infection consists, therefore, in an ability to prevent the invasion and multiplication of the bacteria, and with it the development of poisons which injure the function of the important organs. This immunity mechanism is effective following the subcutaneous inoculation of living bacteria, but it can be readily overcome by the intravenous inoculation of moderately large doses of paratyphoid B bacilli. Overwhelming blood infections of the immunized rabbit produce a cycle of events identical to that described for the typhoid animals. The intoxications provoked by the inoculation of heat-killed paratyphoid bacilli or their extracts have not been investigated, and therefore no attempt is made to explain the mechanism of susceptibility and nonsusceptibility to preformed toxins.

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[67] Jour. Path. & Bacteriol., 1920, 23, p. 171.

[68] Jour. Exper. Med., 1916, 23, p. 601.

From this discussion it is evident that the disease produced in the rabbit by intravenous injection of typhoid or paratyphoid B bacilli must be considered a specific toxicosis. In this term we include the rôle played by so-called endotoxins, and assume in addition that toxic substances (perhaps amines) are produced from the host's protein by some form of proteolysis, which is stimulated by the disintegrated bacteria and their products. The abrupt increase in the nitrogenous excretion (primarily the allantoin fraction) of rabbits injected with living typhoid bacilli, demonstrated by Pepper and Miller,[69] is no doubt a distinct indication of an extensive katabolism of body protein. Resistance to this form of experimental disease is a local or general "antitoxic adaptation immunity" and cellular in nature. The native immunity of rabbits for the typhoid bacillus depends entirely on (1) the ability of the endothelial cells, polymorphonuclear leukocytes, and perhaps the body fluid to destroy the invading bacteria; (2) the mechanism to prevent the formation of foci from which the blood stream is constantly seeded; and (3) the detoxicating power of the liver and the tissues in general. These factors, inherent in the rabbit, can be made inoperative by the injection of a very large dose (over 5,000 million) of typhoid bacilli. An acquired immunity by means of dead or living vaccines can even then prevent the

[69] Jour. Infect. Dis., 1916, 19, p. 694.

final issue produced by the exhaustion of the bonemarrow with its resulting toxemia. Such an acquired resistance is primarily cellular and mainly directed against the liberated toxins. It appears that the actual destruction of the bacteria and the elimination of foci responsible for the continuous invasion of the blood stream is managed by the same, perhaps slightly enhanced, cellular factors responsible for the inherent native immunity.

Susceptibility of the rabbit to paratyphoid B bacilli resolved itself into an inability of certain tissues and their cells, perhaps together with the blood, to kill the introduced bacteria completely. As a result, foci occur in the least resistant tissues (bonemarrow and lymphatic tissues), and the blood stream and organs are gradually invaded with newly developed bacteria. Organs like the liver and spleen, which were able to free themselves to a large extent of living paratyphoid bacilli during the first period of the infection, are then overwhelmed with bacteria and their toxins. Immunity produced by recovery from a slight infection consists of a fairly speedy sterilization of the tissues and blood, together with the elimination of foci, which act as seed beds for the ensuing septicemia in susceptible animals. This is probably not the same mechanism as that evolved by immunization with dead bacteria.

Various questions suggest themselves; for example, Is the immunity produced in rabbits against typhoid or paratyphoid bacilli comparable with the one provoked in man by the prophylactic inoculation of vaccines? From the information at hand it is impossible to answer this important question. Our experiments on guinea-pigs, cats, and monkeys indicate that each species disposes of the intravenously injected organisms of the typhoid-paratyphoid group in its own characteristic way. The immunized guinea-pig destroys typhoid and paratyphoid bacilli more readily than the normal; while monkeys and cats behave like the rabbit. It is quite possible that typhoid fever in man really is a toxicosis, or an endotoxin disease, as proposed by Staddelmann and Wolf-Eisner.[70] The immunity mechanism could then follow either the course outlined for the typhoid or the paratyphoid B infection in the rabbit. It may, however, be entirely different and be more readily comparable with the conditions found in the guinea-pig. The available bacteriologic and immunologic data pertaining to typhoid fever in man are not sufficiently complete to venture these far-reaching deductions based on analogy.

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[70] München med. Wchnschr., 1907, 54, pp. 1161 and 1237.

CONCLUSIONS

A small number of typhoid bacilli inoculated intravenously into normal rabbits of the same litter was rapidly removed from the blood stream at the end of from 10 to 15 minutes after the inoculation. About 20 to 30% of the inoculum were found in the liver; a smaller amount in the spleen, bonemarrow and lungs; and comparatively small numbers in the lymph nodes, kidneys, muscle, etc.

The gallbladder bile contained only a small number of bacteria, while the gallbladder wall received, according to its size, a proportional share of the total number of bacteria deposited in the liver. The intraportal injection presented no advantage over the common intravenous method.

Large numbers of typhoid bacilli are less speedily removed from the blood stream, but the distribution in the organs remains the same in the first 4 to 8 hours.

The bacteria taken up by the tissues are rapidly reduced in number, particularly in the liver, spleen and lungs. The bonemarrow is less active and not infrequently the typhoid bacilli multiply in these tissues and form foci, which reinfect the blood stream. The gallbladder can become infected either as a result of an immediate extensive elimination of the introduced bacteria, or through the continuous discharge of bacilli from liver foci in the hepatic bile, or in consequence of an embolic infarction of the gallbladder capillaries causing a diphtheritic cholecystitis. In the latter instance the cystic bile may not become invaded with typhoid bacilli for 24-48 hours after the intravenous injection.

The distribution and destruction of small or large doses of typhoid bacilli is practically the same in the normal as in the immunized rabbit, whether protected by the injection of dead or living organisms. The only noteworthy difference between the normal and immunized rabbit is found in the lung and spleen, namely, the lung of the immunized rabbit takes up a somewhat greater number of bacteria than the normal; and the spleen of the immune sterilizes itself more slowly than the normal.

Localization in the gallbladder and bile 96 to 120 hours after the infection occurs more readily with moderately heavy suspensions prepared from rabbit-blood agar than those obtained from peptic-digest agar slants.

Rabbits which succumb to the typhoid intoxication regularly harbor enormous numbers of typhoid bacilli in the bonemarrow of the long bones; these foci are probably the seed beds responsible for the continuous invasion of the blood stream and the subsequent overwhelming infection of the liver and spleen.

Paratyphoid A bacilli act in a manner identical to typhoid bacilli. The mechanism of destruction of paratyphoid B bacilli, whether B. aertryckei of rabbit origin, or B. schottmülleri in the immunized and normal rabbit, is different from that reported in the foregoing for the typhoid bacillus. As a rule, paratyphoid bacilli disappear more quickly from the blood and tissues of immunized than normal animals. The activity of the splenic tissue in the destruction of these bacteria is noteworthy and deserves further investigation.

The blood and serum of normal rabbits in vitro is bacteriolytic for typhoid and paratyphoid A bacilli, but is inactive for paratyphoid B bacilli. The serum of immunized rabbits in vitro, whether protected against typhoid, paratyphoid A or paratyphoid B bacilli, is nonbactericidal.

Pieces of excised organs of exsanguinated rabbits heavily infected with bacilli of the typhoid-paratyphoid group when incubated in test tubes at 37 C. may show in the first 2 hours a slight bactericidal action. The property is marked in the excised lung tissues, but not in its extracts and is dependent on the living protoplasm of the cell.

The nature of the immunity of the rabbit against the typhoid and paratyphoid B bacilli is considered in some detail, and it is pointed out that an intravenous injection of a large dose of B. typhosus produces primarily a toxemia, while the invasive B. aertryckei provokes in small amounts a progressive septicemia. The various cellular factors, which, in the light of available knowledge, are responsible in the normal and immunized rabbit for the local and general antitoxic adaptation immunity are discussed.